COLUMBIA RIVER SALMONID OUTMIGRATION:

McNARY DAM PASSAGE AND ENHANCED SMOLT QUALITY

Second Year Completion Report

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EXECUTIVE SUMMARY

We evaluated the effects of the McNary Dam transportation system on
emigrating fall and spring chinook smolts using physiological indices of
stress (e.g., plasma cortisol, hepatic glycogen, leucocrit, interrenal cell
nuclear diameter) and performance tests (e.g., saltwater challenge,
secondary stress challenge, disease resistance). We also conducted
controlled experiments in a hatchery environment to characterize the
fishes' physiological responses to stress, and disease resistance to
allow a basis for judging the nature of the stress experienced by smolts
at McNary Dam. Based on these studies, we concluded that:

- Juvenile fall chinook were stressed by the collection system at McNary Dam.

- The elements of the collection system had cumulative effects on the fishes'
  response to the system.

- Changes in the collection system between 1982 and 1983 decreased the total
  stress experienced by fall chinook collected.

- There were seasonal variations in some physiological responses to stress,
  probably the result of changes in the environment.

- The maximum raceway density of 0.5 $lbs \cdot gal^{-1}$ was not excessive.

- Fall chinook which were anesthetized, handled, and marked were no more
  stressed than fish which just went through the collection system, but
  required a day's recovery time before transport or liberation.

- Optimum length of time for fall chinook to recover from the stresses of
  collection is 24 to 48 h.

- Loading fish into the transport vehicle was the most stressful event in the
  transportation procedure.

- The transport vehicles were not stressful and the fish showed some recovery
  from the stress of loading while enroute.

- The maximum transport loading density of 0.5 lb-gal-1 was not excessive.

TABLE OF CONTENTS

GOAL AND OBJECTIVES

The ultimate goal of this study, as originally stated, was to increase yield (catch and escapement) of salmonids in the Columbia River. Our principal objectives were (1) evaluate the McNary Dam smolt bypass and collection facility; and (2) propose and evaluate methodologies that minimize stress caused by bypass, collection, and handling. These objectives were accomplished by testing the following operational objectives and null hypotheses:

1. Evaluate the stress imposed by passage and collection at McNary Dam.

    1a. Ho: Dam passage and collection is not stressful and certain features (e.g., bar-sorters) encountered by migrants at dams are not more stressful than other features of the bypass and collection system.

    1b. Ho: Recovery time after dam passage and collection is not necessary to ensure optimum smolt quality and performance at **the** time of loading for transportation or at the time of release.

2. Evaluate the cumulative stress imposed by dam passage, collection, and transportation.

    2a. Ho: Fish transported after collection at dams are not more stressed than those either transported directly from hatcheries or allowed to migrate after dam passage.

3. Evaluate the effects of anesthetics, handling, and marking on smolts collected at McNary.

    3a. Ho: Anesthetized and handled fish are not more stressed than fish bypassed or collected at dams.

3b. Ho: Recovery period for optimum performance for fish
         anesthetized at dams is not longer than the time
         needed to regain swimming activity.

4. Evaluate means of alleviating the severity of stress from bypass,
    collection, handling, and transportation.

    4a. Ho: Severity of stress does not differ during the course of
             the smolting cycle.

    4b. Ho: Loading density at McNary holding facilities is not
             important in determining performance of fish.

    4c. Ho: The density at which fall chinook are transported from
             McNary Ham to Bonneville Dam is not important in
             determining performance of fish.

INTRODUCTION

The construction of hydroelectric dams on the Columbia River and its main tributary, the Snake River, has greatly altered this ecosystem and affected the anadromous salmonids in the system. Dams have reduced the river flow (Raymond 1979, Bentley and Raymond 1976), increased water temperature (Bentley and Raymond 1976), and increased gas supersaturation (Ebel and Raymond 1976). Completion of the last four dams on the Columbia and Snake Rivers resulted in a two- to three-fold increase in the time required for salmonid smolts to migrate downriver (Raymond 1968, 1969, 1979), and it has been hypothesized that this has increased the exposure to predation and pathogens and has increased residualism among smolts (Bentley and Raymond 1976). Moreover, environmental modifications in the Columbia River system have been beneficial to native and exotic predators (Maule and Horton, in press; Stainbrook 1983; Gray et al. 1984) and, perhaps, competitors (Hjort et al. 1981). These factors have resulted in reduced survival of salmonids (chinook salmon and steelheads) migrating from the upper Snake River, such that in years of very low water flow, survival to The Dalles Dam may be as low as 5% (Raymond 1979). Ultimately, this reduced survival of emigrants is reflected in as much as a 10-fold reduction in percent of emigrants returning as adults (Raymond 1979).

Columbia River hatcheries have increased production as part of a strategy to increase numbers of returlng adults. In the mid-1960's, the annual Columbia River system smolt migrations were estimated to be 3 to 5 million fish of wild origins (Raymond 1979). In 1984, 19 million smolts, mostly hatchery production, are expected to pass McNary Dam (James Athearn, U.S. Army Corps of Engineers, Walla Walla, Washington, personal comm.).

Various management strategies have been aimed at modifying the dams to eliminate gas supersaturation problems, divert fish away from turbine intakes, and bypass fish downstream of the dam. Additionally, smolts are now collected at the dams, transported in trucks or barges, and released below Bonneville Dam, the last downstream barrier on the Columbia River (Delarm et al. 1984). Transportation of some salmonid species has shown positive results when compared to non-transported migrants, but the percent of smolts returning as adults remains low (Park et al. 1983).

The collection and transportation of smolts places physiological demands on the fish which may result in reduced fitness to perform activities necessary for survival (Schreck 1981). In this study, we evaluate the collection and transportation system at McNary Dam (Fig. 1) to determine which elements of the system are the most stressful to emigrating salmonids, and to propose methodologies to minimize the impact of those stressful elements. Our conceptual framework in the study was based on the model proposed by Schreck (1981, 1982) in which an organism's ultimate capacity to perform physiological tasks, or performance capacity, is determined by the environment and stress, which can act physiologically and psychologically to reduce performance capacities. For example, as smolts migrate downstream, they have individual abilities or capacities to resist disease or avoid predators. When these fish encounter the stresses of a hydroelectric dam, their ability to perform these tasks, or performance capacity, may be reduced.
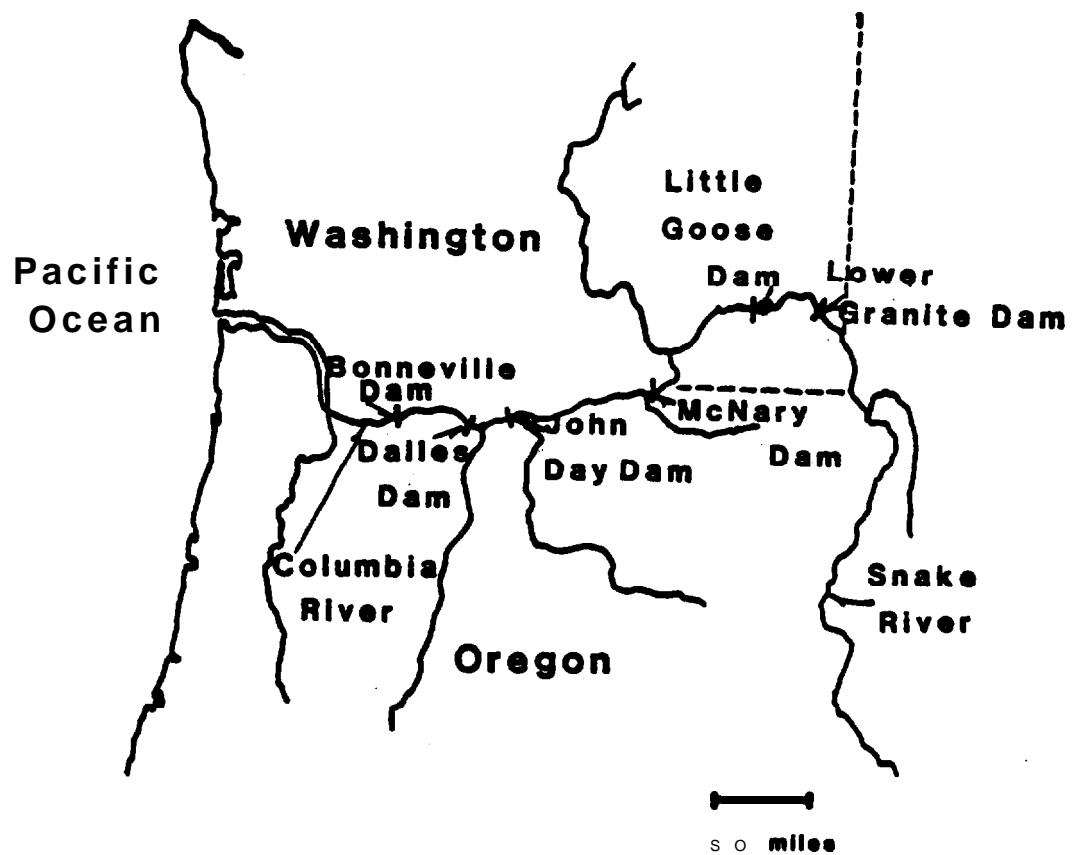
Figure 1.   The mid and lower Columbia and Snake Rivers.

In this study, we collected emigrating salmonids from various points within the collection and transportation system and monitored various physiological indices of stress (e.g., plasma cortisol, hepatic glycogen, among others). At the same time, we subjected fish to several secondary challenges (i.e. saltwater, secondary stress, disease) to measure their actual performance capacities. Thus we describe the fishes' physiological status and the effects of passage through the system, or some handling procedure on the ability of the fish to perform tasks relevant to survival.

Although this report takes the form of a completion report, the study continues for a third year under contract to the U.S. Army Corps of Engineers. While this report summarizes the first two years of data and our conclusions to date, complete statistical analysis and recommendations await finalization of sample collection, assay, and analysis.

GENERAL METHODS

During the course of this study, methods and experimental design were changed to fit the needs of the particular investigation. However, certain aspects of the methods were the same throughout the study and these methods are presented here.

Clinical Indices of Stress

Sample collection. Whenever fish were collected for plasma or tissue samples, they were immediately transferred by dipnet into a bucket containing 200 $mg \cdot L^{-1}$ tricaine methanesulfonate (MS-222). It has been shown that at this level, MS-222 does not significantly alter plasma cortisol levels (Strange and Schreck 1978) or other physiological variables (Black and Conner 1964; Houston et al. 1971). This method has been successfully used for rainbow trout (Barton et al. 1980) and was verified by Barton (unpublished data) for juvenile fall chinook by placing fish in 200 $mg \cdot L^{-1}$ MS-222 and serially sampling them through 30 min in one experiment and 45 min in another. There were no systematic differences in plasma cortisol in either experiment.

As soon as fish were immobilized in MS-222, we severed the caudal peduncle and collected blood in heparinized 0.25 ml capllary tubes. Blood samples were centrifuged, and the plasma was removed and stored at -20 C. Additional blood was taken for hematocrit and leucocrit determination (McLeay and Gordon 1977), and blood smears were made for blood cell counts. We also removed livers which were stored in cool 30% KOH, and head kidneys which were stored in 10% phosphate-buffered formaldehyde.

Sample analyses. Hematocrit and leucocrit were recorded at the site. Blood smears were air dried, fixed with 95% ETOH and, at a later date, stained with Giemsa stain. Following the method of Weinreb (1958), we examined the smears at 1000x magnification and counted the number of white blood cells (WBC) found within the area of 300 erythrocytes. WBC identification was based on descriptions of Anderson (1974) and Yasutake and Wales (1983). Thawed plasma was assayed for cortisol using a radioimmunoassay (Redding et al., in press), glucose using the O-toluidine method (Hyvarinen and Nikkila 1962 as cited by Wedemeyer and Yasutake 1977) and lactate using a fluorimetric, enzyme reaction (Passonneau 1974). Hepatic glycogen was assayed by a phenol-sulfuric acid method (Montgomery 1957) that was previously modified and verified in our laboratory for salmonids. Dimensions of interrenal cell nuclei were determined from photomicrographs of Harris' hematoxylin and eosin stained, six-micron-thick sections. The slides were viewed using a fixed-position projector and screen such that the interrenal cells were projected at 1,769x magnification. Length and width measurements of 20 nuclei were averaged to get the score for each fish. Proximate analysis of whole-fish body composition was obtained by drying whole fish and determining fat content with a Goldfisch ethanol fat extractor, and ash content by incineration at 500 C in a muffle oven; an estimate of protein was obtained by subtracting fat and ash from dry weight. Plasma and gill filaments were taken during each sampling period. As indices of smoltification, 'we determined plasma thyroxine using the radioimmunoassay method of Dickhoff et al. (1978); gill Na-K ATPase activity was determined for us by Dr. W. Zaugg, National Marine Fisheries Service, Cook, Washington.

Performance Challenges

Osmoregulatory challenge. During each sampling period in 1982 and 1983, fish at McNary Dam and those transported to Bonneville Dam were collected and exposed to sublethal osmoregulatory challenge. Normal plasma Na levels of smolts in freshwater are between 140-150 $meq \cdot L^{-1}$. When a smolt is put in salt water, plasma Na increases to as high as 210 $meq \cdot L^{-1}$. The smolt's ability to decrease plasma Na is a measure of that fish's osmoregulatory capacity which will decrease if the fish is under stress. In 1982, fish were rapidly transferred from the site of collection to buckets containing salt water (Marine Environments, Inc.). The fish and salt water were then poured into 38 L aquaria, also containing salt water. A salt concentration of 15.0 + 1.0 parts per thousand was selected after a bioassay established this salt level as being tolerable by the salmon. Compressed oxygen was bubbled into each aquarium. The aquaria were in a flow-through water bath so that ambient river temperature was maintained. After 24 h in salt water, all fish were bled, and plasma was collected and stored at -20 C. Total plasma sodium (meq. L^{-1}) was assayed with a flame photometer. In 1983 the SW challenges were changed, in that spring chinook or fall chinook were held in dark plastic buckets containing 20.0 + 1.0 ppt salt water, and the challenges ended after 18 h. These changes were made to eliminate the transfer procedure, increase the osmotic challenge, and reduce the time allowed for recovery. Samples collected in 1983 were assayed using a Na-K analyzer.

<u>Secondary stress challenge</u>. The secondary stress consisted of capturing fish in a dipnet and holding them **out** of the water for 30 s. We 'do not believe that this is a stress that smolts would normally encounter., but rather we used it as a uniform challenge which will help to clarify the degree of stress previously encountered by the fish. We used this same standardized stress in our stress characterization studies. During 1982,. the secondary stress test was conducted on three groups of fish which had been in a raceway for 0, 1 or 7 d, and fish which had been transported to Bonneville Dam. During each sampling period in 1983, we challenged fish which had been in the raceway for less than 4 h, fish from the gatewell, and fish transported to Bonneville Dam. In each instance, after the stress, the fish were released into 100 L tanks with flow-through water and were serially sampled through 24 h.

Hatchery and Emigrating Fish as Controls

On May 28, 1982, approximately 150 fall chinook from Priest Rapids Hatchery were transported to McNary Dam and were placed in a large metal tank (ca. 0.6 x 0.6 x 1.8 m) with flow-through river water (see: Disease Challenge for detai ls of the transportation). An additional 150 fish were held in a 1.2 m circular Fiberglass tank with flow-through water at Bonneville Dan. These fish were to serve as controls, to establish baseline nesting levels of the clinical indices of stress and baseline performance capacities for the challenge tests. After being held **for** 2 weeks, the fish appeared to be in worse condition than fish coming through the co l lection system. Daily nortalities were high and many fish had

fungus. Approximately 2 weeks prior to our sampling in July and August 1982, 150 fall chinook were removed from the holding raceway at McNary Dam and held in conditions as described above. An additional 150 fish were removed from a transport truck at Bonneville Dam and also held. We reasoned that in 2 weeks these fish would acclimate to the holding facilities and could serve as controls for the June and July sampling. These fish also suffered high mortalities and obviously did not adapt to captivity. We did obtain plasma and tissue samples from these fish and assayed these for clinical indices of stress. The results confirmed that these fish did not acclimate. Those results are not included in this report so as not to confuse the results of the other sampling. Similarly, fall chinook from Priest Rapids Hatchery were transported to the OSU Marine Science Center to be controls for the disease challenge and seawater growth experiments. Although these fish were used in the experiments, their very poor quality and survival, independent of the experiments, negates their value as controls.

SYSTEM EVALUATION

Experimental Rationale and Methods

Collection facility. The McNary Dam collection system is intended to divert

emigrating smolts away from the turbines and shunt them to the downstream

side of the dam. Here they are either returned immediately to the river,

or held prior to being transported and released below Bonneville Dam.

Fish encountering the dam are diverted into turbine intake gatewells by

submersible traveling screens (Fig. 2). From the gatewell, fish are shunted

through sluiceways and pipes with various water velocities and pressures to

the downstream side of the dam. Included in this system is a vertical pipe

approximately 12-m long by 0.5 m diameter. On the downstream side of the

dam, fish surface in an upwelling-box, cross a perforated, stainless steel

plate which reduces water volume, and encounter a bar-sorter which is spaced

such that smolts pass between the bars while adult fish and trash continue

to the end of the bars and return to the river. The bars are positioned

just below the water level of the encompassing metal box (ca. 5,800 L).

Fish voluntarily move from this box to another system of pipes and

sluiceways through which they can be diverted to a raceway, the subsampling

system (see below), or back to the river. Modifications of specific

portions of this system occurred between 1982 and 1983 smolt emigrations and

are outlined in Delarm et al. (1984).

Fall chinook smolts were collected at McNary Dam June 14-24, July 7-16,

August 2-11, 1982; and June 14-19, July 7-13, August l-4, 1983. Spring

chinook were sampled during May 3-6 and May 23-26, 1983. We monitored

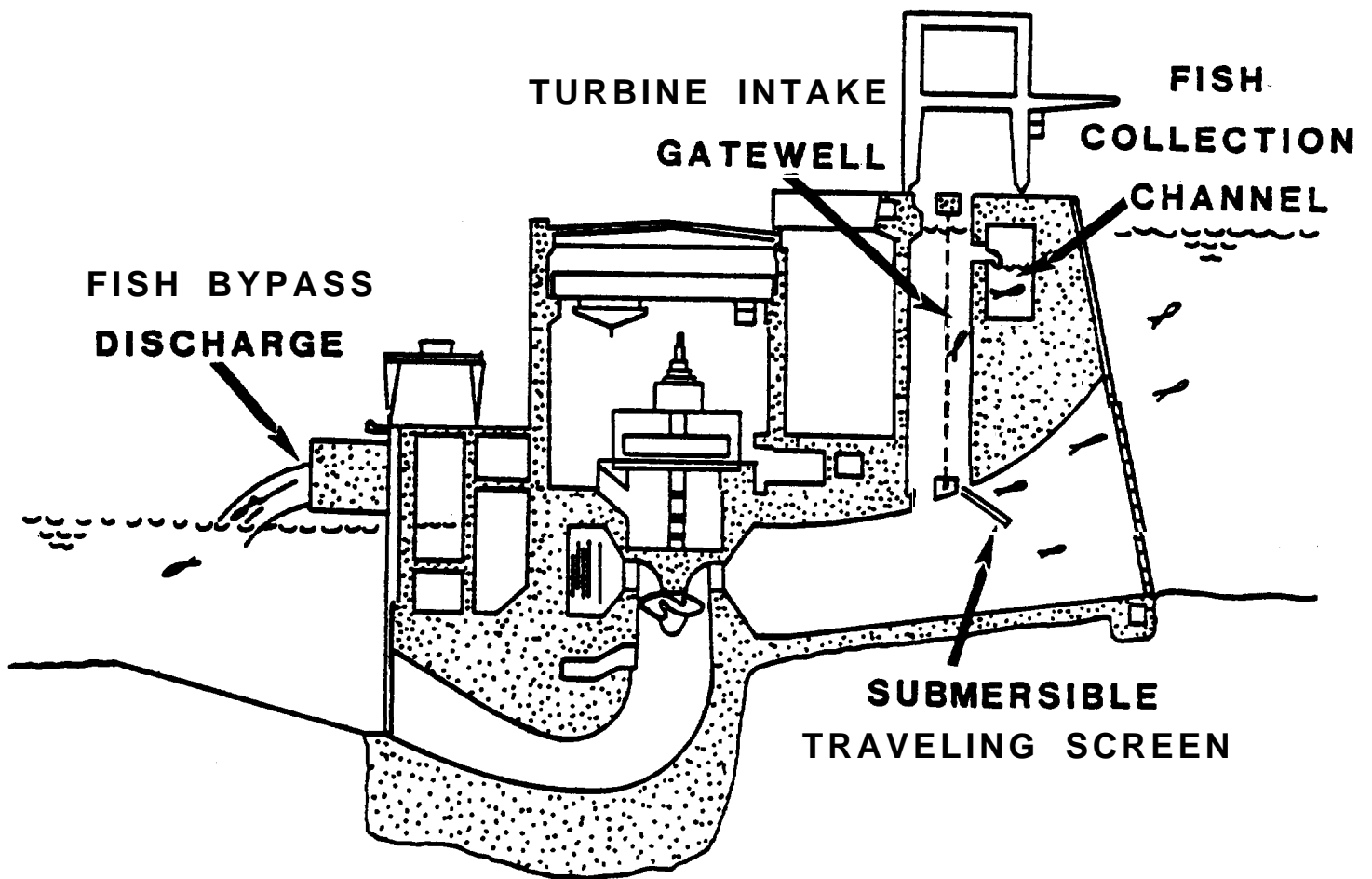changes in degree of smoltification and changes in the environment.

Figure 2. Cross section through a typical dam powerhouse, showing
various elements of the fish bypass system (from: Smith
and Wold 1982).

However, we did not monitor place of origin, i.e. stock differences, of fish sampled. During each sampling period, we collected plasma and tissue samples to monitor clinical indices of stress and we measured smolt's performance in challenge tests. The first place we collected smolts was the turbine intake gatewell, using the gatewell dip-basket technique described by Bentley and Raymond (1968). At the gatewell, the fish have been minimally manipulated by the system. After the fish passed to the downstream side of the dam, they were collected just before the bar-sorter, as they entered the raceway, and at various time points after they entered the raceway. In 1982 we sampled fish after they had been in the raceway for 1, 2, 4, and 8 d. Based on our 1982 results and to examine the short-term effects of holding fish in the raceway, in 1983 we sampled fish which had been held for 1, 3, 6, 12, 24, 48, and 72 h. In order to conduct valid tests, the raceways needed to be filled to normal operational fish density, but it was necessary that all fish entered the raceway at approximately the same time so that we would know how long a given fish had been in the raceway. For example, during the early and late portions of the runs, it took over 24 h to fill a raceway to a density of 0.25 lb-gal-'
(0.03 kg*L-1) which is one-half the maximum allowable limit. To solve this problem, in 1982 we loaded as many fish as possible for a maximum of 4 h, at which time we crowded the fish to one end of the raceway and put a barrier screen in the raceway. This proved unsatisfactory because it required additional manipulation of the fish, and we had mechanical problems with the barrier screen. In 1983 we constructed a 3 x 6 x 10 ft
(0.9 x 1.8 x 3.0 m) livecage of PVC pipe covered on all sides but the top with 0.125-inch (3.17 mm) knotless nylon mesh netting. When suspended

in the raceway, the livecage encompassed approximately 790 gal (3,000 L) of water, and fish were collected directly into it via the established collection system.

As another indication of degree of stress encountered in the system, in 1983 we examined recovery rates of fish collected at the various sampling points. Fish taken from before the bar-sorter and the raceway were held in small (ca. 25 L), dark plastic buckets furnished with continuous flow-through river water, 12 fish per bucket, Large (ca. 100 L), dark plastic tanks with flow-through river water, 100 fish per tank, were used for fish taken from gatewell and transport vehicles (see: Transport Evaluation below) as it was necessary to collect a large number of fish at one time at these stations. Fish thus collected were serially sampled through 24 h.

Raceway density evaluation. It has been shown that the density at which fish are held can have physiological consequences (Fagerlund et al. 1981; Patino and Schreck, unpublished data); in fact, holding fish at extremely high densities (i.e., confinement in a dipnet or small livecage) has been used as a chronic stressor (Strange and Schreck 1978; Barton et al. 1980). In addition to our regular raceway sampling in 1982 and 1983, during August 12-14, 1982, we examined several raceway densities at McNary Dam to determine the optimal density to hold emigrant fall chinook (Objective 4b). We had intended to repeat this in July 1983; however, the vagaries of the fish run prohibited this replication (i.e., too many fish in the system in July and too few in August). As has been indicated, it was difficult to obtain enough fish through the collection facility on any single day to adequately assess the effects of various raceway loading densities. During

the August 1982 tests, we simulated various densities by first loading the raceways to a given weight of fish and then crowding them so that the fish weight available to raceway volume was either 0.50, 0.25, or 0.13 lb*gal*-1 (0.06, 0.03, 0.015 kg*L-l), 0.5 $lb \cdot gal^{-1}$ being the maximum production value in use. Since as many as 24 h was required to fill the raceways, fish at the time of crowding had been in the raceway from 0 to 24 h. We designated these samples as Crowded, t = 0. The fish were also sampled 24 h later (t = 24 h).

Anesthetic, handling, and marking. The primary tools used on the Columbia River to study emigration patterns and to evaluate the efficacy of management practices, such as transporting smolts, is the marking of smolts by cold-brand and the insertion of coded wire tags (CUT) into smolts' snouts. Marking fish requires that they be anesthetized and handled, and it is assumed that upon release these fish behave and survive similarly to the general smolt population. In order to determine if anesthetized and marked fish are stressed more or less than fish just collected at the dam (Objective 3a) and to determine optimum time for recovery from the marking procedures (Objective 3b), we investigated this system on July 12-14, 1982, and July 10-14, 1983. As indicated in the description of the collection facility, after fish have passed below the bar-sorter, they can be diverted for marking. From the bar-sorter, fish pass along a sluiceway to a large, stainless steel box (ca. 7,000 L). The water in this box flushed the fish through electronic counters and into a larger holding tank (ca. 13,750 L). Each day, the fish accumulated during the previous 24 h were crowded and then dipnetted from the holding tank and passed, via a stainless steel chute

into an anesthetic bath containing MS-222 at a concentration of

approximately 50 $mg \cdot L^{-1}$. Workers examined the anesthetized fish and

recorded species, degree of descaling, and presence of brands. Unmarked,

non-descaled fish (fall chinook at the time of our study) were shunted to

the marking stations in a flow of anesthetic-laden water. At the marking

station, workers clipped off the fish's adipose fin, applied a freeze brand,

and inserted a CWT. Marked fish were shunted in fresh water to a raceway or

directly into a transport-truck tank. We collected fish from the holding

tank, the anesthetization bath, and after they were fully marked (adipose

fin clipped, branded, and CWT). In 1982 we held fully marked fish in a

large plastic tank (ca. 100 L) with flow-through water, and sampled them

after 24 and 48 h to determine recovery rates. In 1983 we collected fish

which had been fully marked with a brand and CWT and fish which had received

a clipped adipose fin and brand, but not a CWT. These groups were held in

similar plastic tanks and were serially sampled through 72 h.

Results and Discussion

Collection facility_. Smolts passing through the collection facility are

stressed, apparently in a cumulative manner, by the elements of the system.

In 1982, cortisol levels increased as fish progressed through the system,

until some time after they reached the raceway where the cortisol levels

peaked and returned to relatively low levels within 4 d (Fig. 3). Cortisol

levels in fish held for 8 d in the early run were elevated (Fig. 3),

indicating that holding fish beyond 4 d might be counterproductive. The

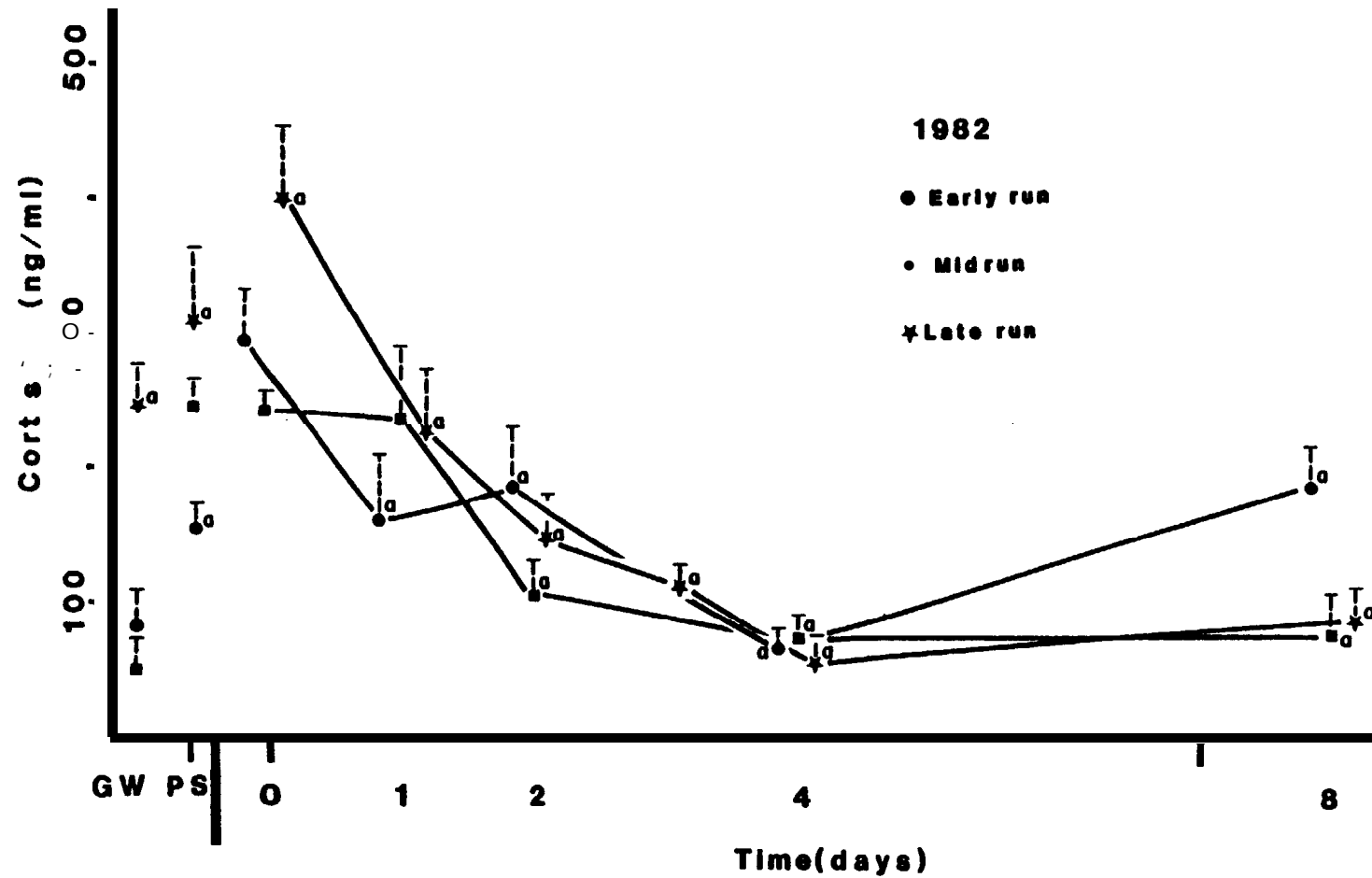late-run fish had significantly higher cortisol levels in the gatewell,

Figure 3.   Plasma cortisol levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell (GW), before passing the bar-sorter (PS) and during 8 d of raceway recovery.   Sampling was conducted on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982. All points represent mean + SE for n = 10 to 13, except GW for June sample, n = 5.   Values marked (a) are significantly different from Time = 0 of same line (P < .05 Least Significant Difference test, LSD).

pre-sorter and early raceway samples which we attributed to the large number
of adult American shad in the collection system. Water temperature was high
during the late run (21-22 C); however, our characterization studies show
that acclimation temperature does not influence plasma cortisol dynamics
(see <u>Acclimation temperature and stress</u>). During the 1983 late run, we were
unable to sample smolts from the gatewell because of the large numbers of
shad present relative to few smolts. However, our results from other parts
of the system (Figs. 4-6) do not show elevated cortisol in the late 1983
sampling, even though water temperature was elevated.

The peak plasma cortisol levels of fall chinook sampled in 1982 are
generally higher (300-400 $ng \cdot ml^{-1}$) than the levels in fish from 1983
(225-250 $ng \cdot ml^{-1}$). This may reflect the change in our raceway holding
procedure between the two years (i.e., mechanical crowding vs. livecage).
The magnitude of the plasma cortisol elevation appears to have an effect
on the time required for plasma cortisol to return to pre-stress levels
(recovery time). Using cortisol levels in fish from the gatewell, we
defined plasma cortisol levels of 100 $ng \cdot ml^{-1}$ or less as the baseline for
fish in the system. When plasma cortisol levels were highest (300-400
$ng \cdot ml^{-1}$), it took as long as 4 d for them to return to the baseline
(Figs. 3 and 4); however,when the peak levels were lower (200-250 $ng \cdot ml^{-1}$),
plasma cortisol returned to the baseline within 24 h (Figs. 5 and 6).

There were no consistent patterns in cortisol response through the smolt
runs. In 1982, the highest absolute cortisol values and highest relative
changes in plasma cortisol were from the late-run fish (Fig. 3), while in
1983 the highest values occurred during the early run (Fig. 4). This
variability could be the result of variation in the environment or the fish.
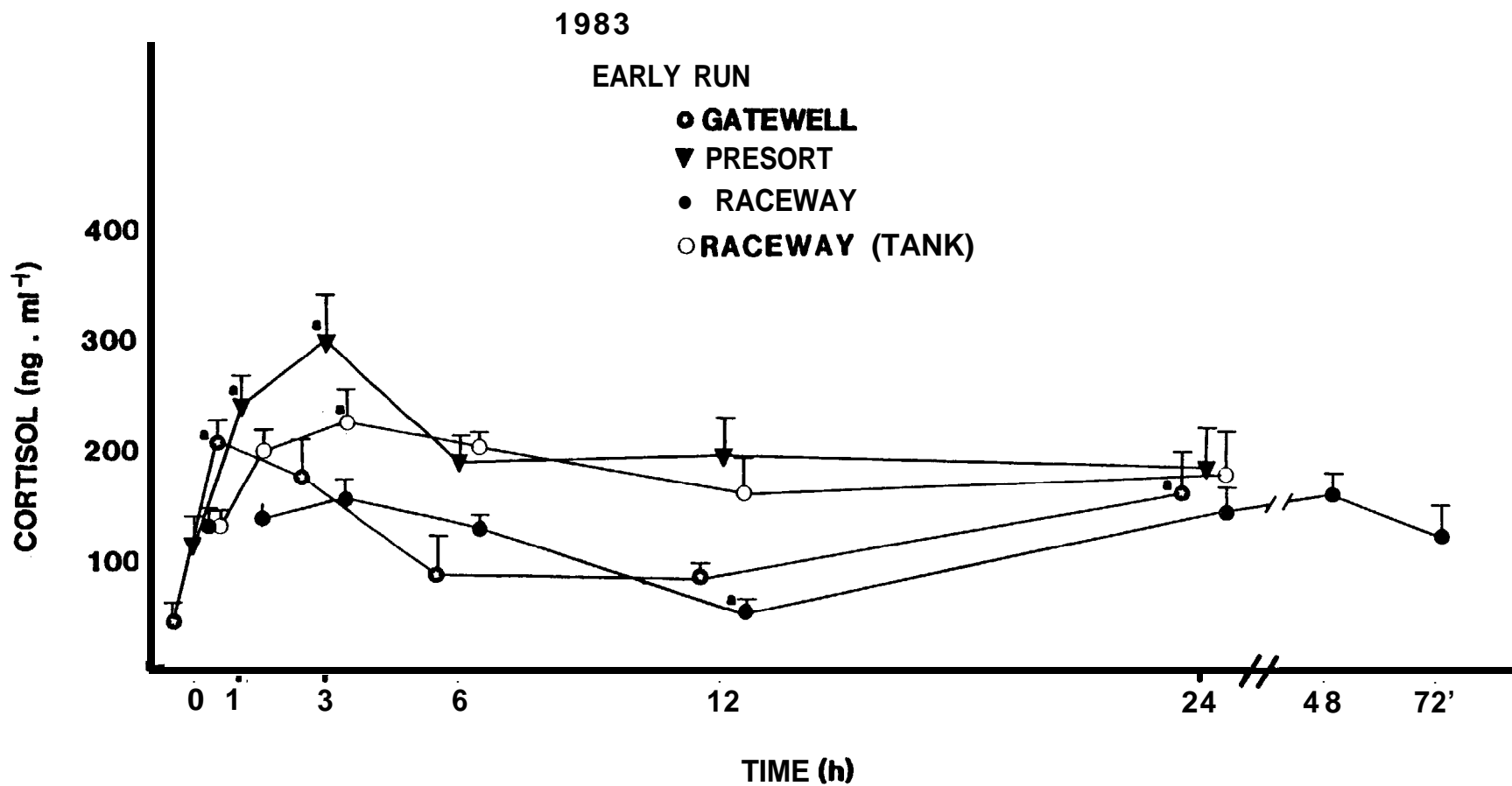
1983

EARLY RUN
- ○ **GATEWELL**
- ▼ PRESORT
- ● RACEWAY
- ○ **RACEWAY (TANK)**

Figure 4.   Plasma cortisol levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell, before passing the bar-sorter (presort) and raceway. Fish from the gatewell and presort were held in large, opaque plastic tanks (ca. 100L) and serially sampled through 24 or 72 h during June 14-19, 1983.   All points represent the mean + SE for 6 to 12 fish.   Values marked (a) are significantly different from Time = 0 of same line (P < .05, LSD test).
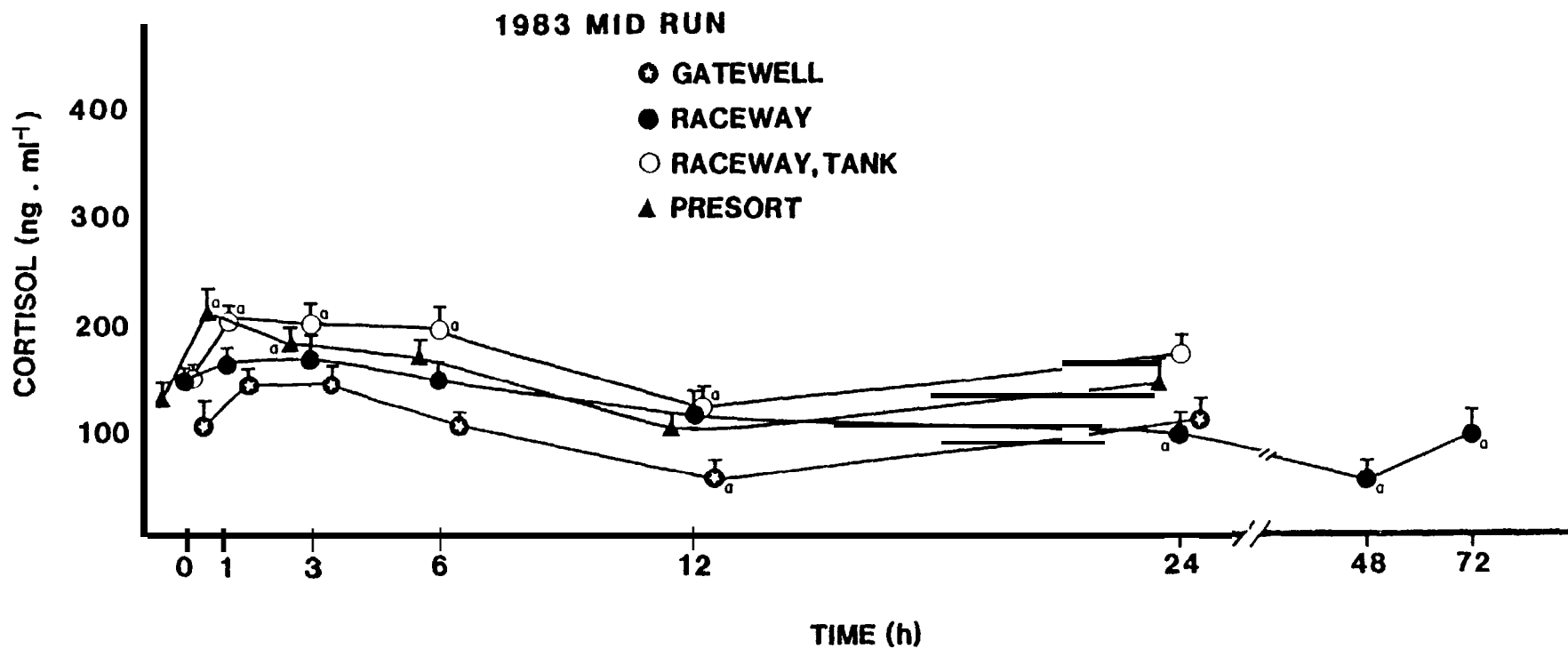
Figure 5. Plasma cortisol levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell,
before passing the bar-sorter (presort) and raceway. Fish from the gatewell and preseort were
held in large, opaque plastic tanks (ca. 100L) and serially sampled 24 and 72 h during July 7-13,
1978. All points represent the mean + SE for 10 to 12 fish. Values marked (a) are significantly different
from Time = 0 of same line (P < .05, LSD test).
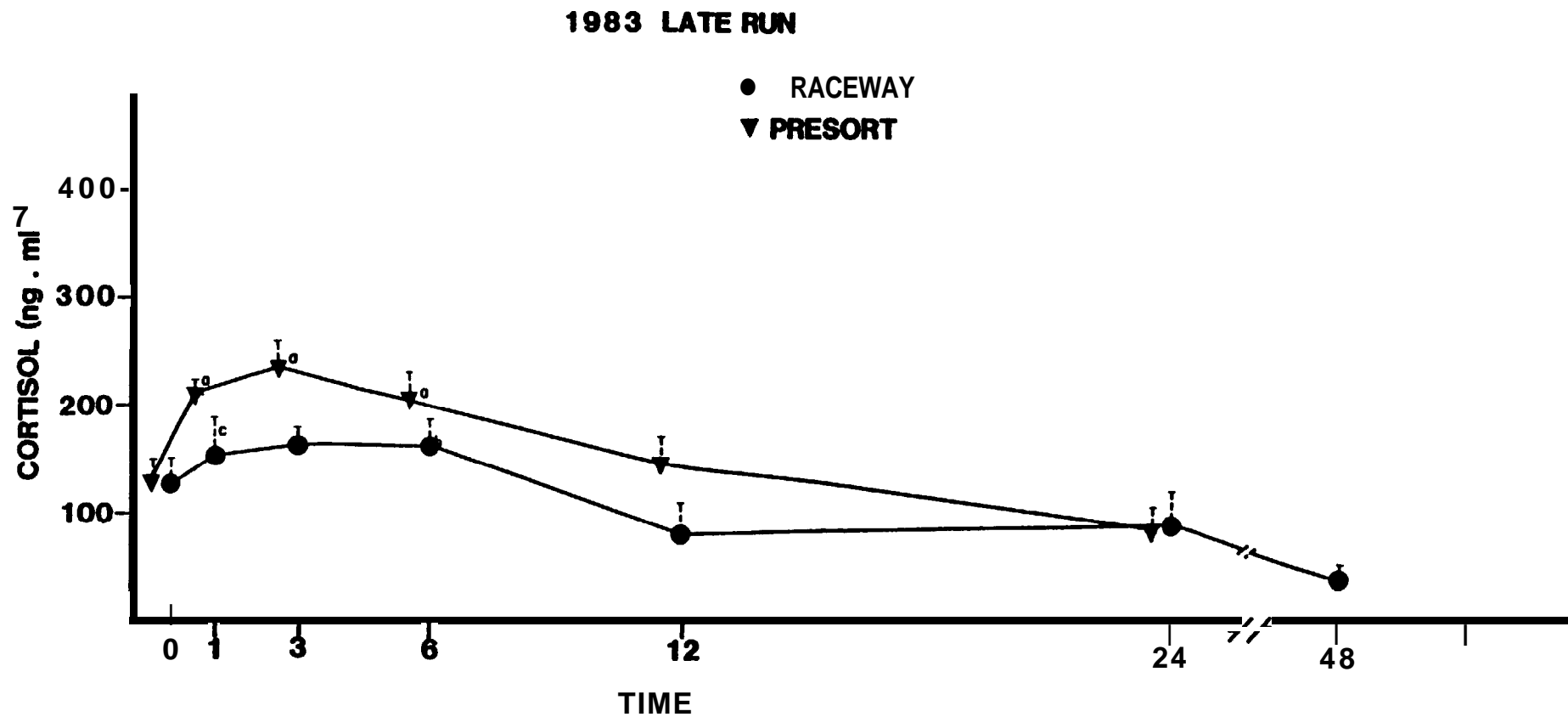
1983 LATE RUN

● RACEWAY
▼ PRESORT

Figure 6. Plasma cortisol levels of juvenile fall chinook salmon sampled from the McNary Dam, before
passing the bar-sorter (presort) and raceway. Fish from the presort were held in large,
opaque plastic tanks (ca. 100L) and serially sampled through 24 or 72 h during August 1-4,
1983. All points represent the mean + SE for 6 to 12 fish. Values marked (a) are significantly
different from T = 0 of same line (P < .05, LSD test).

We found no differences in weight, length (Fig. 7), gill Na-K ATPase or thyroxine (Fig. 8) between 1982 and 1983 which could account for this variability. Water temperature and river velocity are two environmental factors which showed variability between the two years (Fig. 9); however, there does not appear to be a correlation between these environmental factors and variability in the plasma cortisol response (also see: Acclimation temperature and stress).

There was no significant change in interrenal nuclear diameters in fall chinook collected at McNary in 1982 (Fig. 10), indicating that the stresses encountered at the dam are not of a long enough duration to cause chronic mobilization of cortisol synthetic mechanisms. Interrenal cells actively manufacturing cortisol have a hypertrophied nucleus as the result of RNA synthesis. In acute stress, however, cortisol will be released without fully mobilizing the synthetic process.

Hepatic glycogen, which may be converted to glucose as an energy source during stress, was highly variable in fall chinook. In 1982, hepatic glycogen tended to decrease in fish collected in the system and through 4 d in the raceway when glycogen levels were barely detectable (Fig. 11). The 1983 sampling only extended through 3 d, but despite variability, it appears that glycogen is decreasing through the holding period (Fig. 12). The glycogen levels in fish at the dam are close to the levels in hatchery fish after 20 d of fasting (see: Nutrition and Stress), and the high variability is, speculatively, the result of some smolts not feeding after release from the hatchery. Subjectively, we recall many more empty stomachs than full stomachs in fish dissected at the dam.
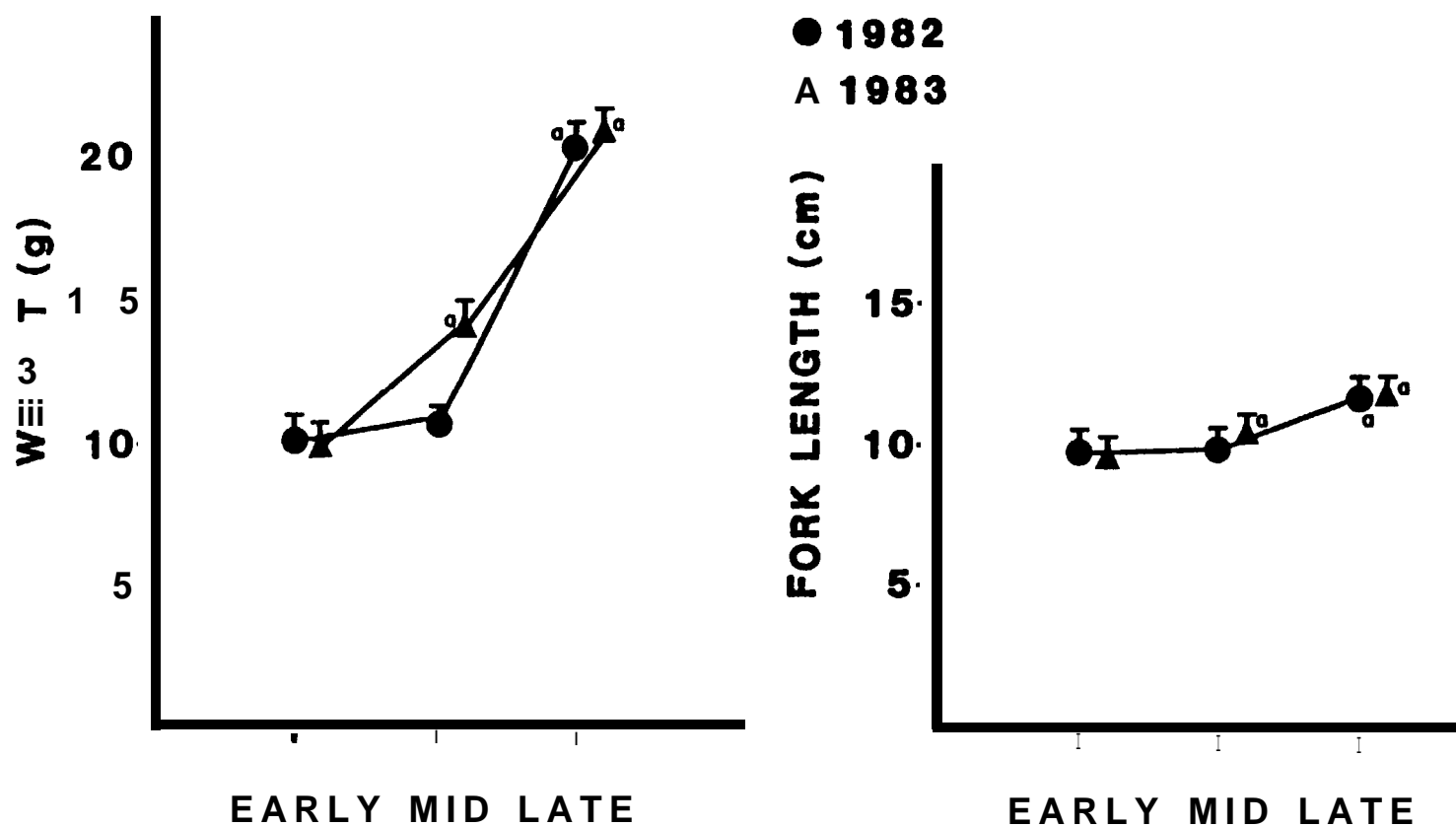
Figure 7.   Weights and fork lengths of juvenile fall chinook salmon collected at McNary Dam on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982, and at similar times in 1983.   All points are means + SE for 100 fish.   Values marked (a) are significantly different from other values of the same year (P < .05, LSD test).
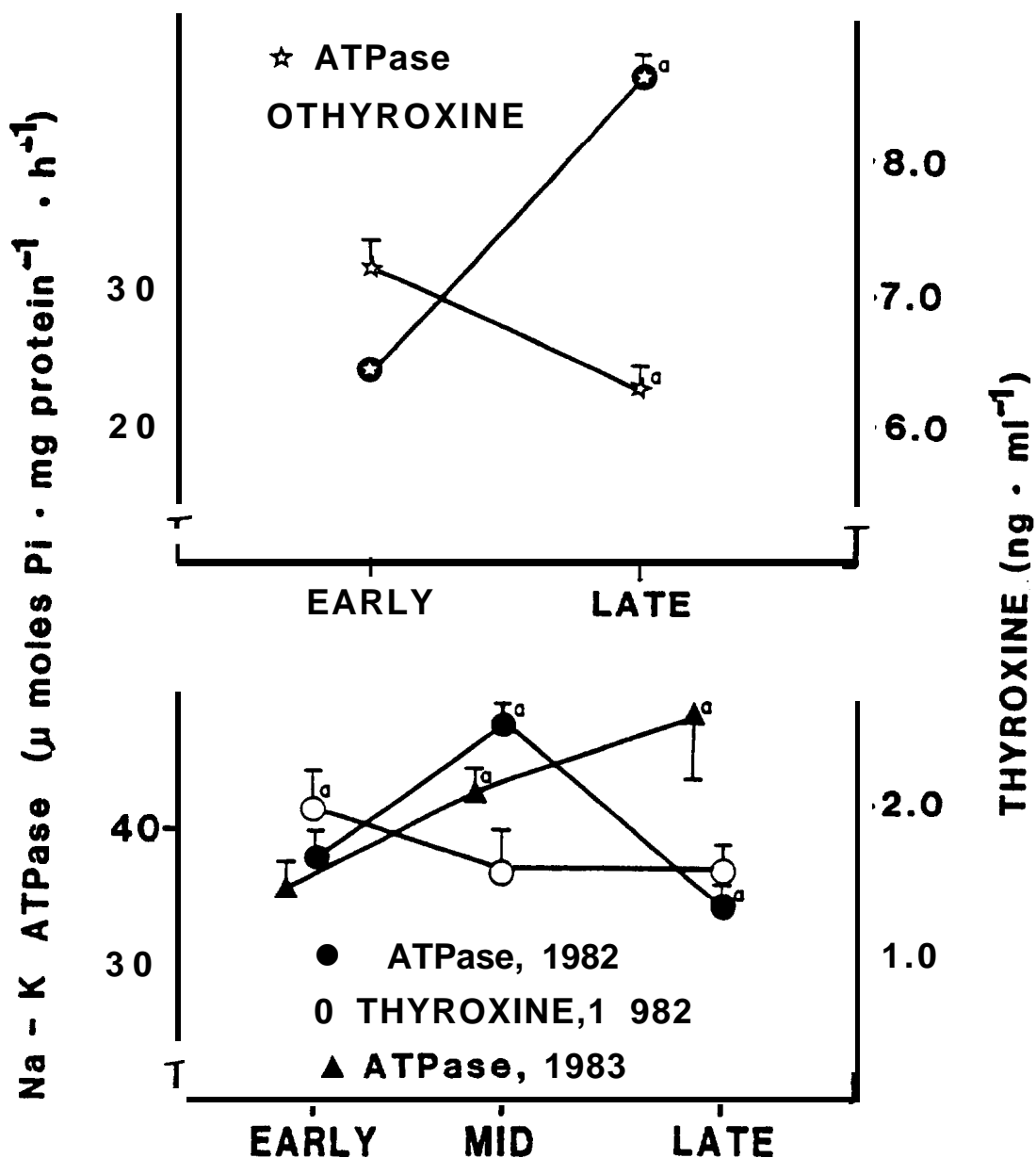
Figure 8. Indices of smoltification (Na-K-ATPase activity and plasma thyroxine levels) for juvenile fall chinook salmon collected at McNary Dam on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982, and similar times in 1983 (bottom); and for juvenile spring chinook salmon collected at McNary Dam on May 3-6 (early) and May 23-26 (late), 1983 (top). All points represent means + SE for 15 to 30 fish. (Na-K-ATPase activity was assayed by Dr. W. Zaugg, NMFS, Cook, Washington.) a = significantly different from all similar variables of the same year (P < .05, LSD test).
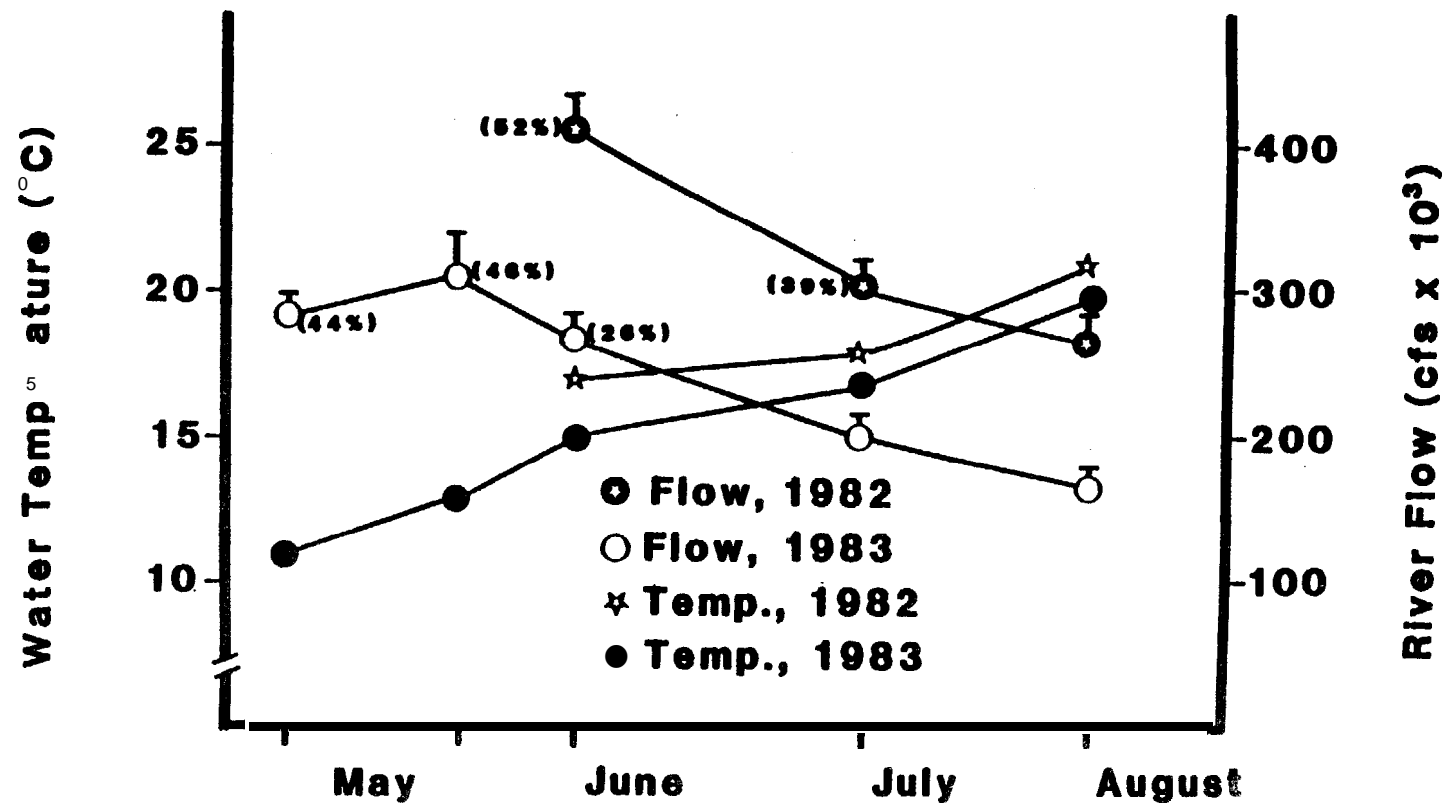
Figure 9. Water temperature and average flow (mean daily flow + SE) of the Columbia River at McNary Dam for May, June, July, and August, 1982 and 1983. Numbers' in parentheses are the average percent of total flow which went over the dam spillway. (River flow data is from Basham et al. 1983 and Delarm et al. 1984.)
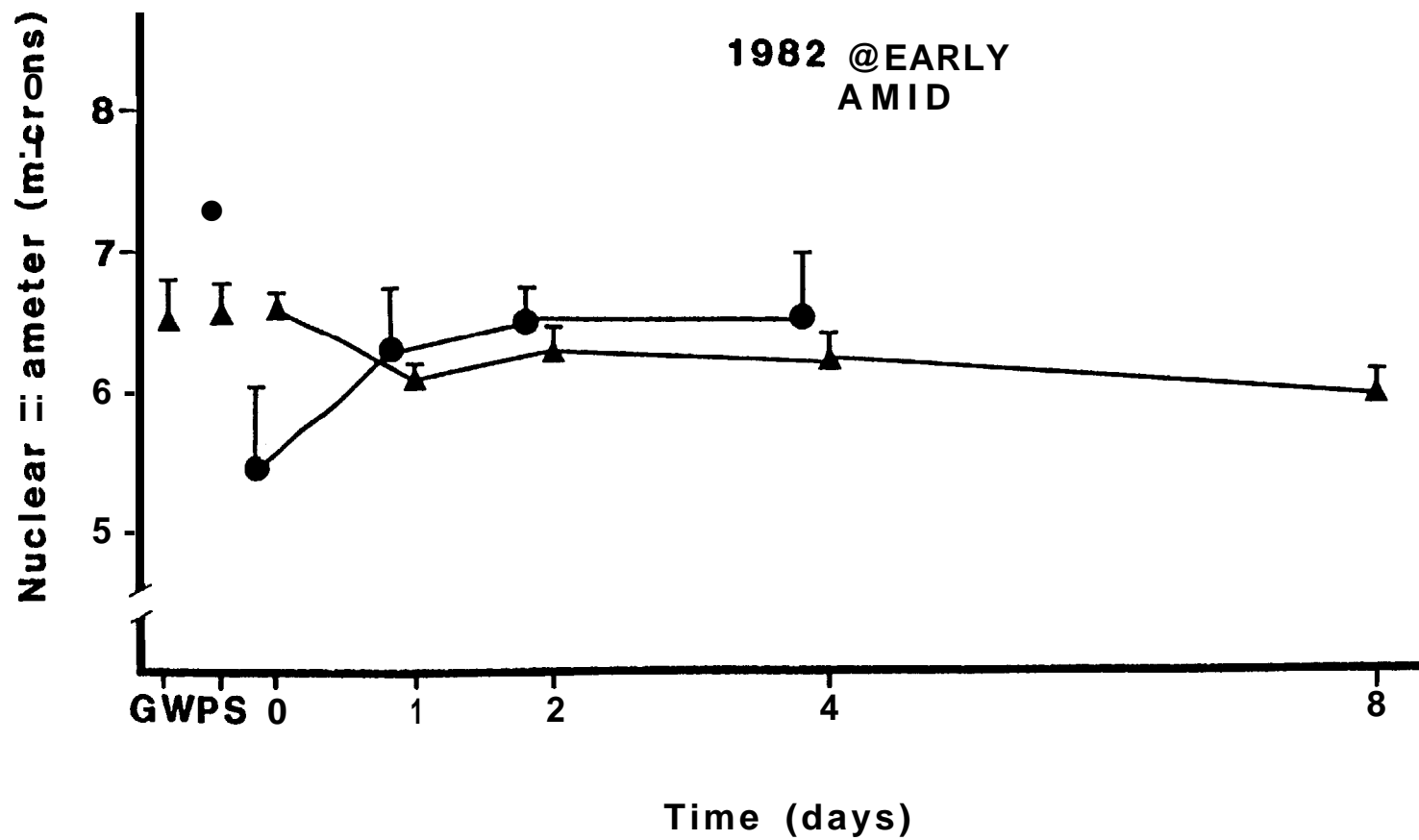
Figure 10. Interrenal cell nuclear diameters of juvenile fall chinook salmon collected from the gatewell (GW), before the fish pass the bar-sorter (PS), and for up to 8 d in a raceway at McNary Dam. Samples were collected June 14-24 (early) and July 7-16 (mid), 1982. All points are the means + SE for 4 to 6 fish.

Figure 11. Hepatic glycogen levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell (GW), before passing the bar-sorter (PS), and during 8 d of raceway recovery, Sampling was conducted. on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982. All points represent mean + SE for n = 5 or 6.
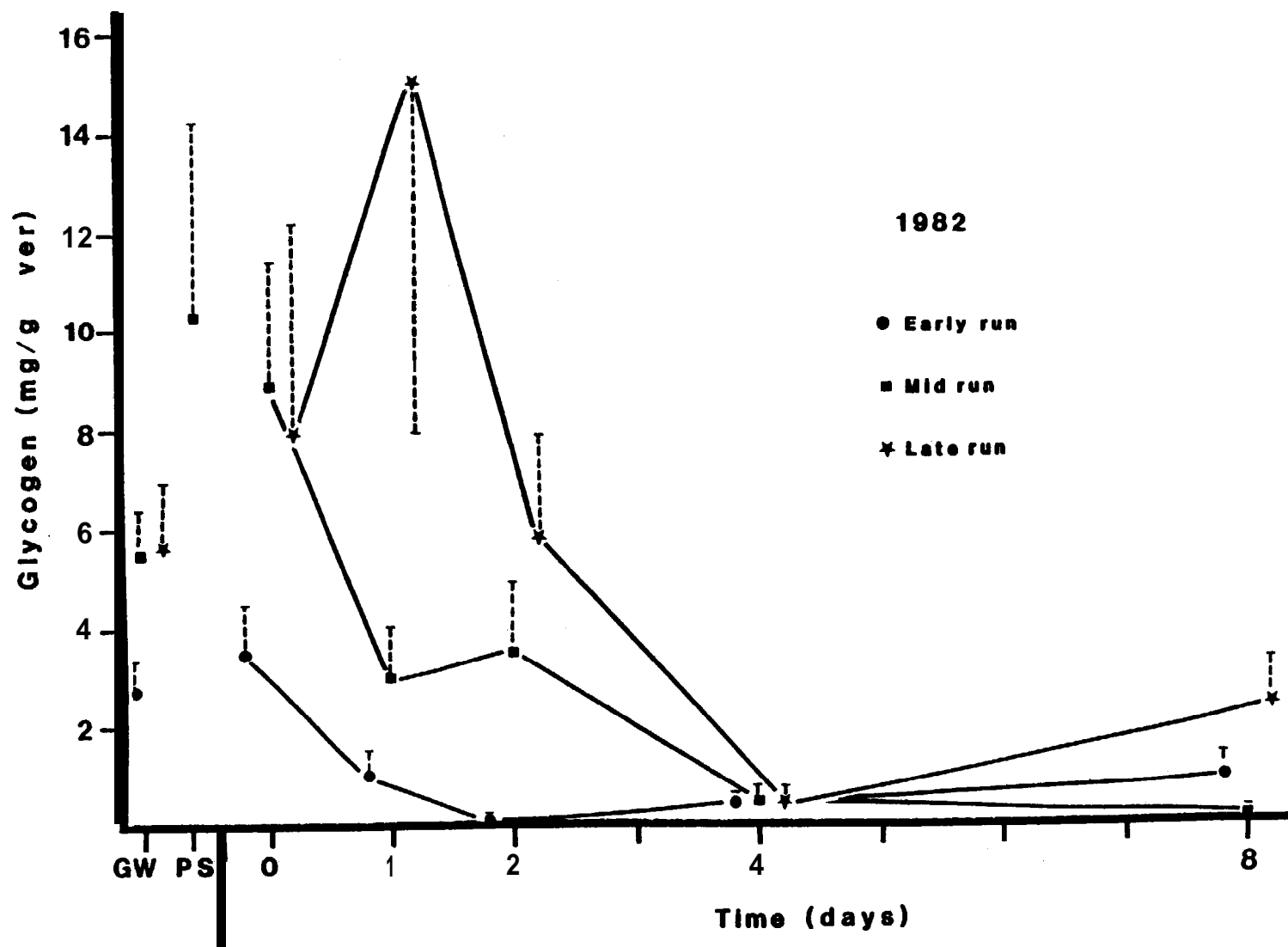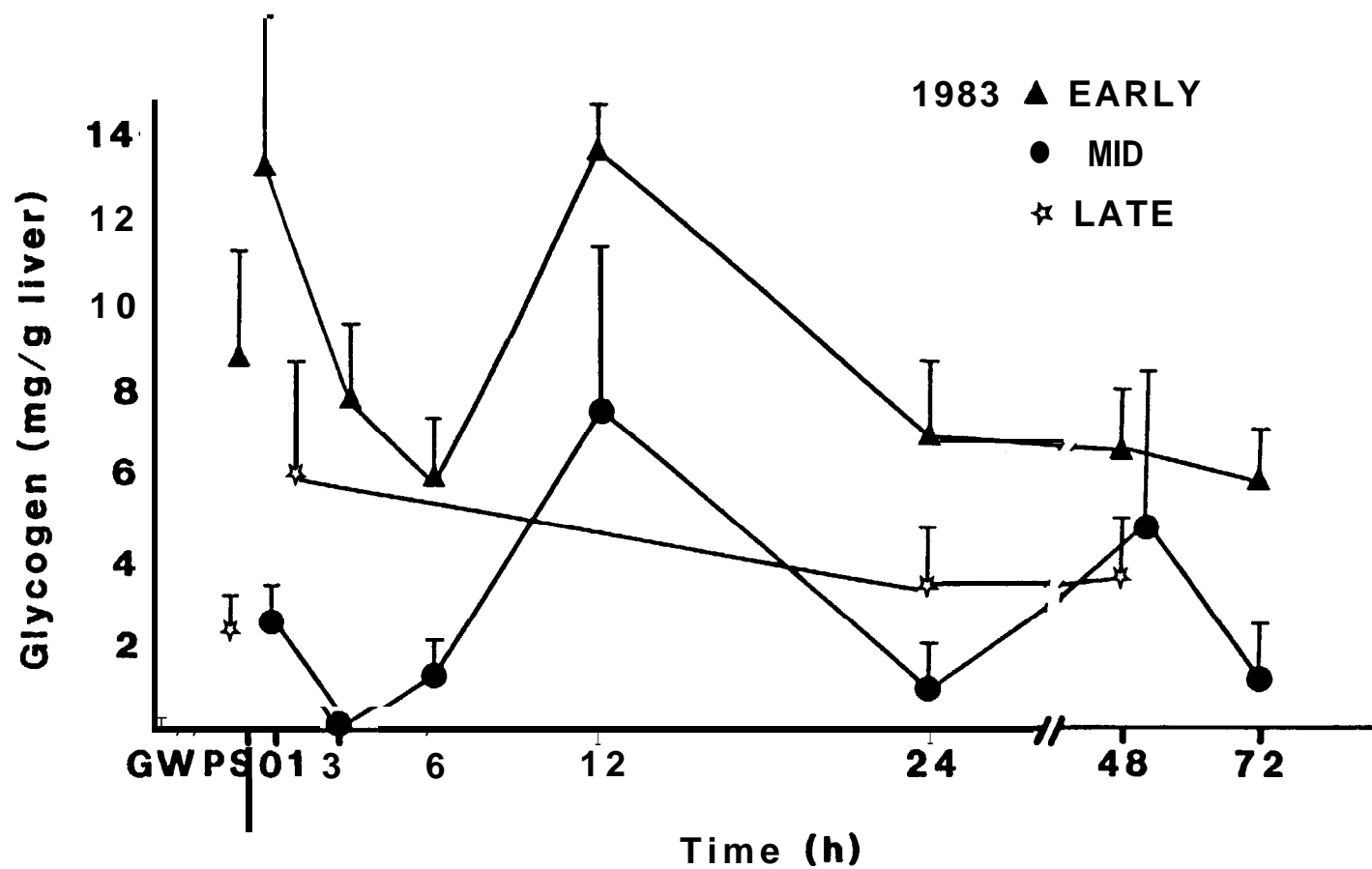
Figure 12. Hepatic glycogen levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell (GW), before passing the bar-sorter (PS) and during 8 d of raceway recovery. Sampling was conducted on June 14-19 (early), July 7-13 (mid), and August 1-4 (late), 1983. All points represent mean + SE for n = 5 or 6.

Hematocrits of fall chinook had little variability at McNary Dam in
1982 (Fig. 13); mean values remained close to 50% throughout the sampling.
In 1983, however, the hematocrits were lower (ca. 40-45%) in samples from
the gatewell and presort, and increased in samples from the raceway,
especially in the mid-run sample (Fig. 14). Leucocrits, did not show a
consistent pattern of response in fall chinook (Figs. 13 and 15). However,
the numbers of WBC relative to numbers of erythrocytes on blood smears was
significantly depressed 24 to 48 h after fall chinook (Figs. 15 and 16)
entered the raceway. The reduction in WBC may be the result of the
increased cortisol (Figs. 3-6) which can have cytolytic or redistributional
effects on the WBC (Baxter 1976).

Proximate analysis of body composition revealed an increase in percent
fat and a decrease in percent moisture in fall chinook as the run progressed
in 1982 (Fig. 17). However, there were no detectable changes in body
composition which could be attributed to the stress of collection or
t ransportatlon.

Osmoregulatory capacity of fall chinook decreased in fish collected in
the later run of 1982 (Fig. 18) and 1983 (Fig. 19A). Furthermore, there was
a decrease in osmoregulatory capacity if fish were held in a raceway for 8 d
(Fig. 18).

The secondary stress test demonstrated the cumulative effects of the
stresses fish encounter in the collection facility. Plasma cortisol reached
progressively higher levels and required longer time to return to pre-stress
levels in fish which had been in the raceway for 0 to 20 h when compared to
fish transported to Bonneville Dam or fish that had been in the raceway for
7 d (Fig. 20). Also of note is that fish in the raceway for a short time
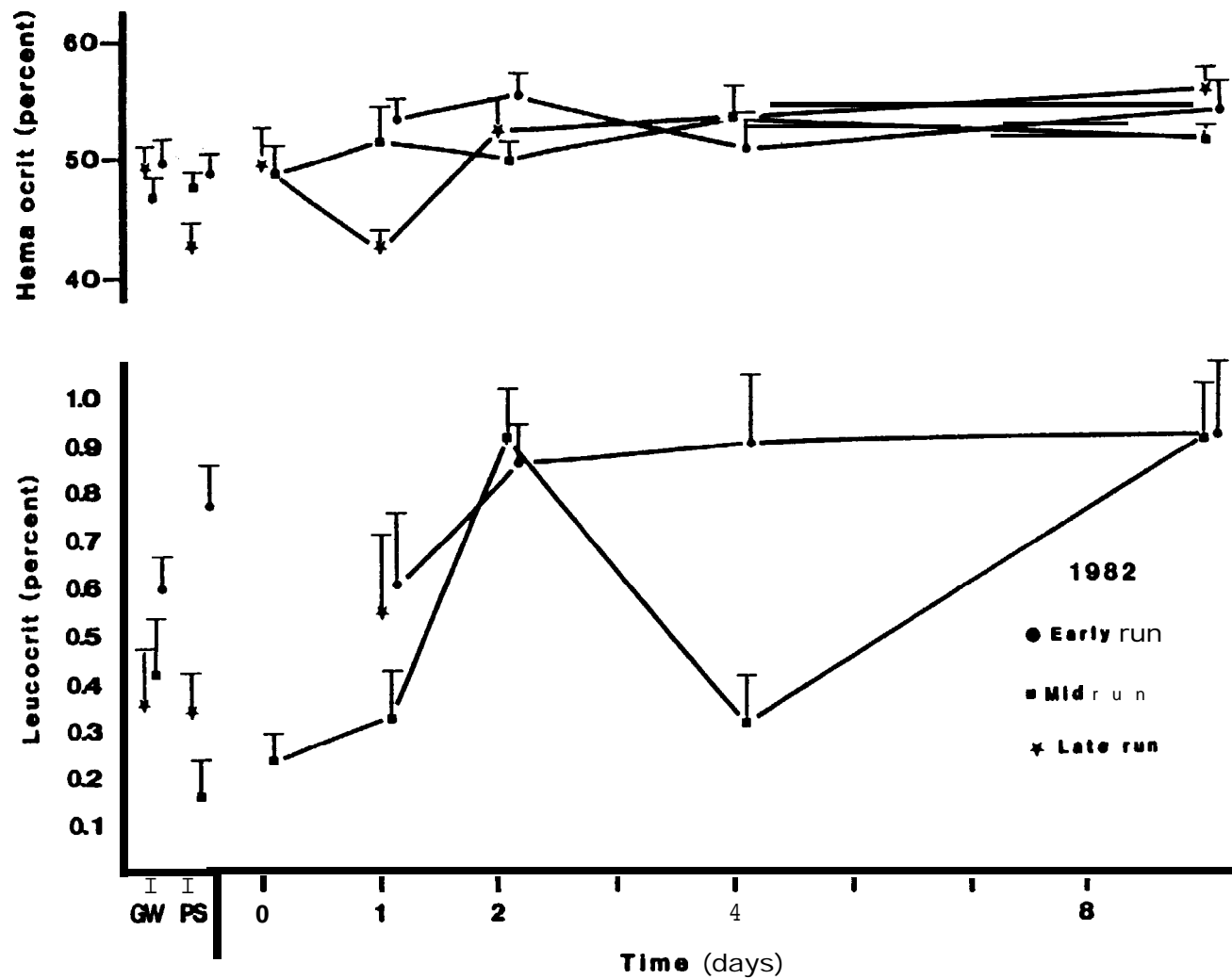
Figure 13. Leucocrit and hematocrit values (mean + 1 SE) for juvenile fall chinook salmon sampled from the gatewell (GW) and pre-sorter (PS) and through 8 d of recovery at McNary Dam. Each point represents 6 fish taken on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982.
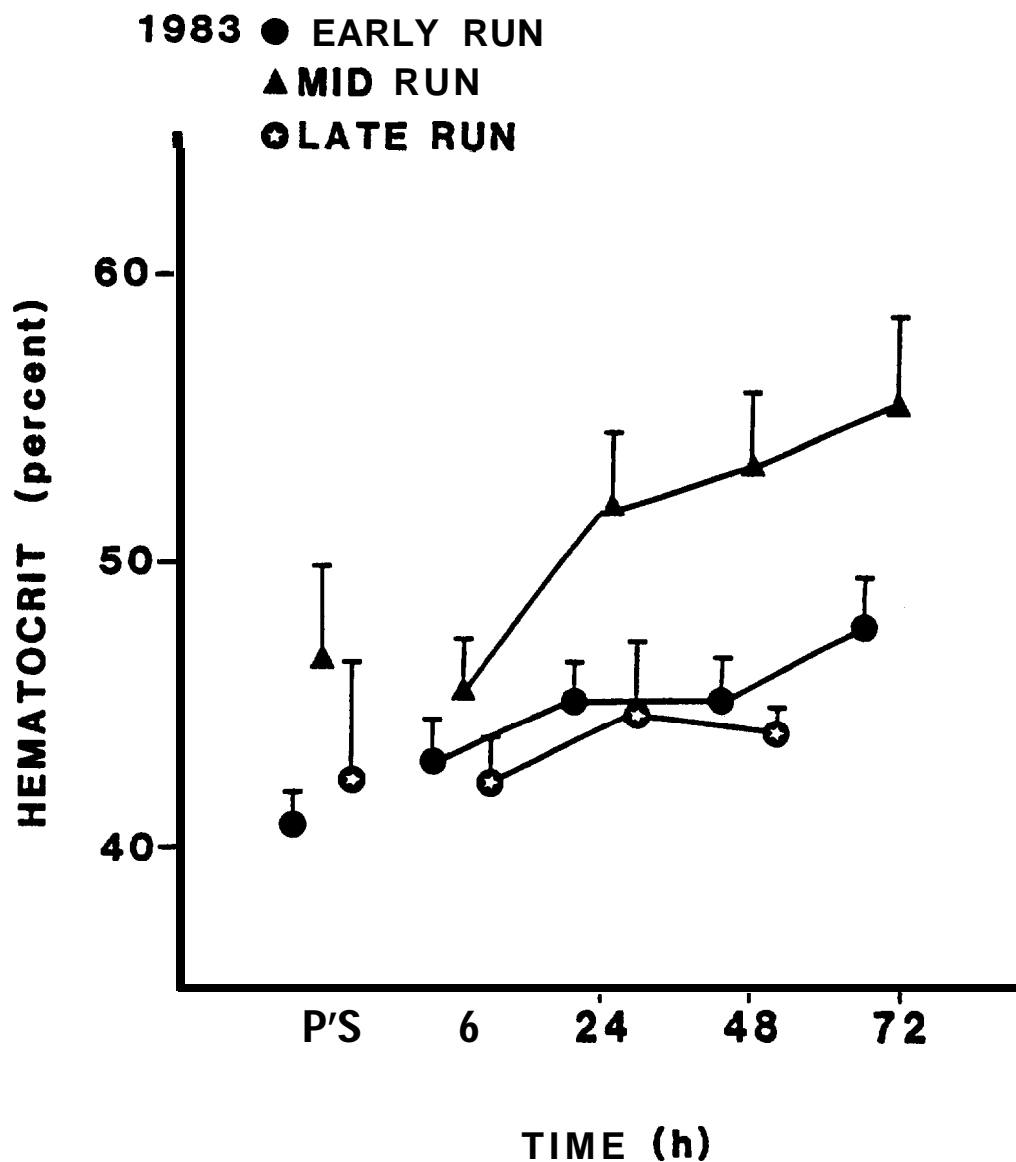
Figure 14. Hematocrit values for juvenile fall chinook salmon collected before they cross the bar-sorter (PS) and during various times in the raceway at McNary Dam during June 14-19 (early), July 7-13 (mid), and August 1-4 (late), 1983. All points are the means + SE from 6 fish.

1983
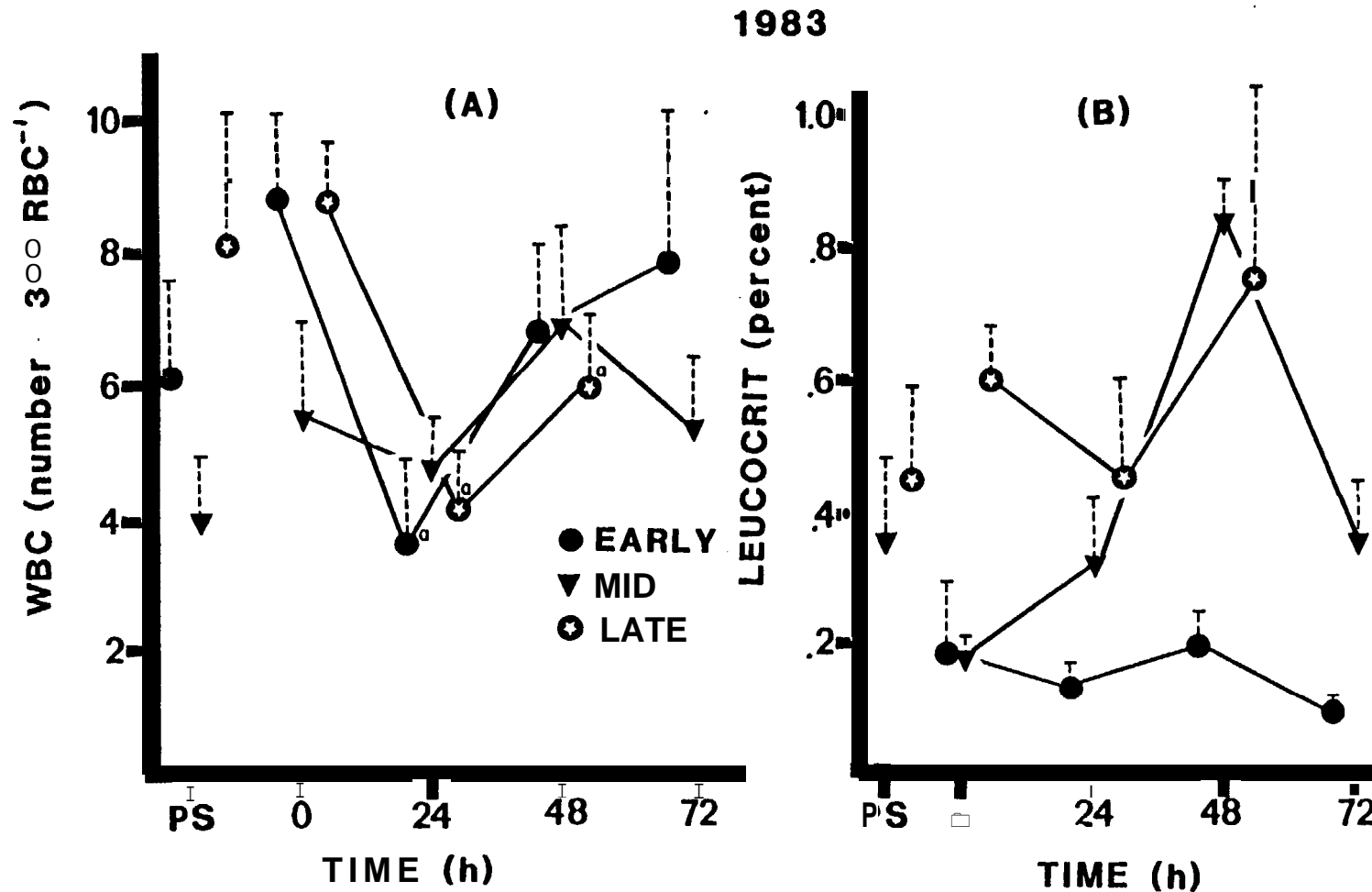
Figure 15. White blood cell (WBC) counts (A) and leucocrit values (B) for outmigrating fall chinook salmon sampled from just before the bar-sorter (PS) and after various recovery times in the raceway. WBC points are the means + 1 SE of the average of two replicated counts of the number of WBC's among 300 erythrocytes (RBC) on blood smears from 6 fish. Leucocrits are means + 1 SE for the same 6 fish. Points marked (a) are significantly different from Time = 0 of same line (P < .05, LSD test).

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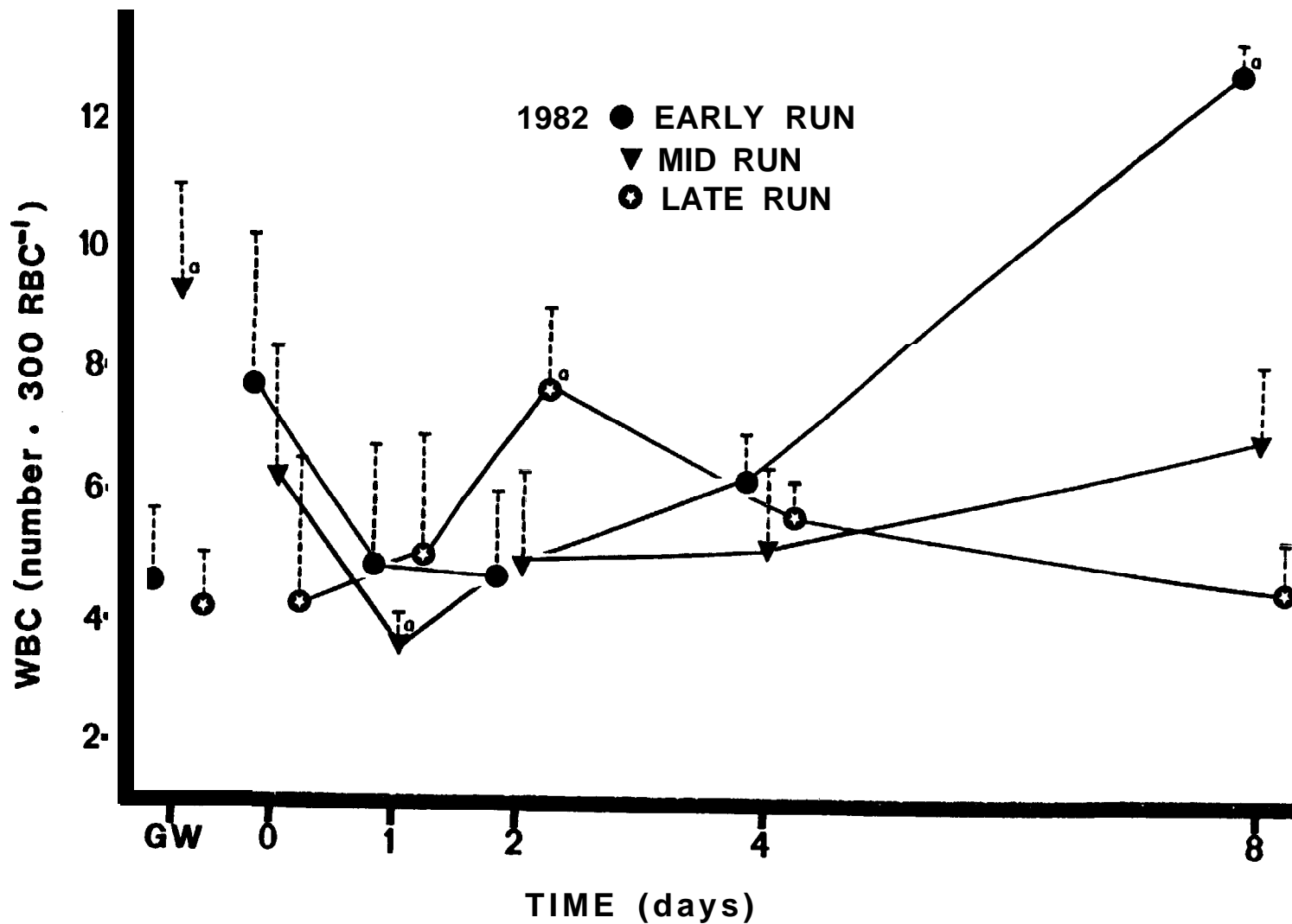
Figure 16. White blood cell (WBC) counts for outmigrating fall chinook salmon collected from the gatewell (GW) and after various recovery times in the raceway at McNary Dam. Samples were collected June 16-24 (early run), July 14-22 (mid run), and August 2-10 (late run), 1982. All points are means + SE of the average of two replicate counts of the number of WBC's among 300 erythrocytes (RBC) on blood smears from 6 fish. Points marked (a) are significantly different from Time = 0 of same line (P < .05, LSD test).
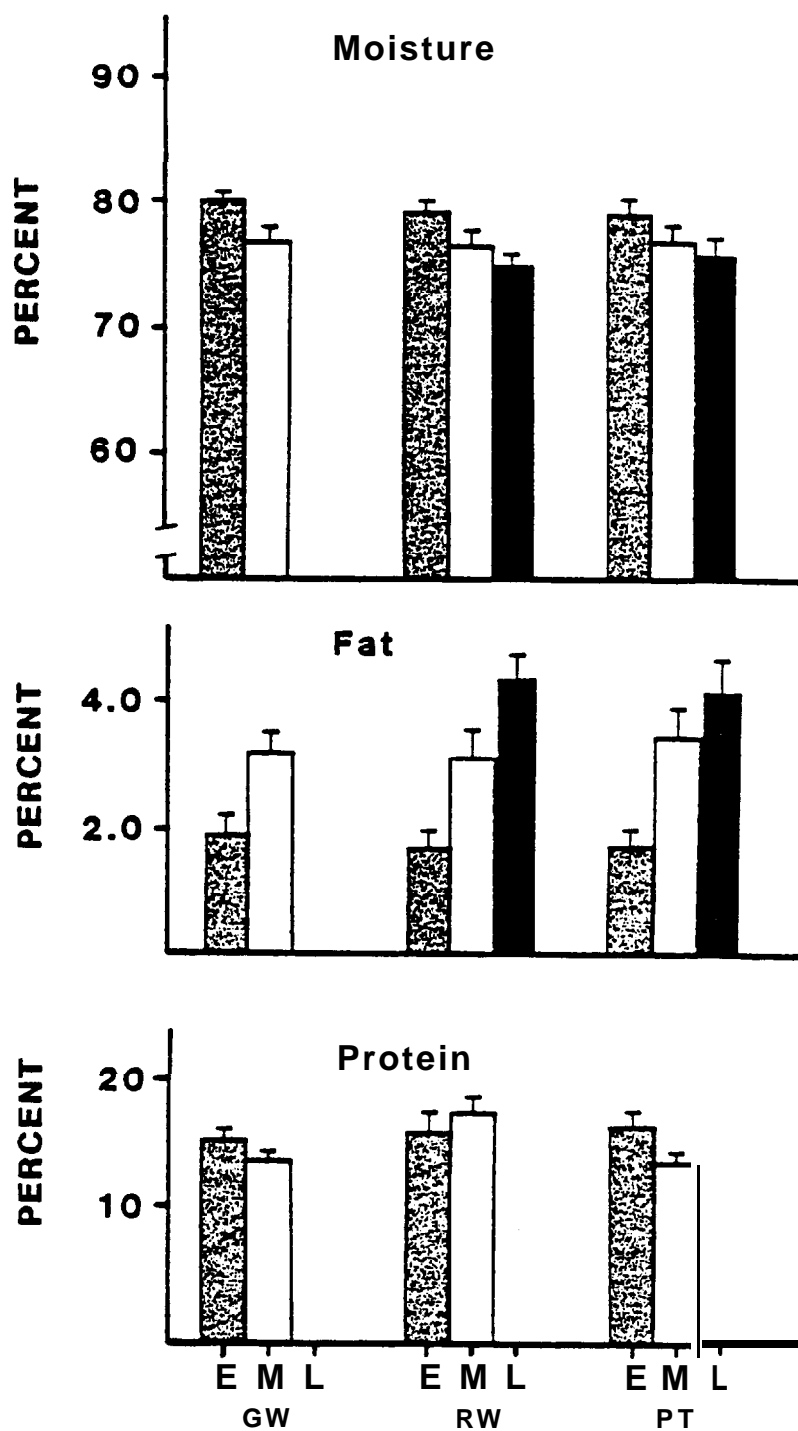
Figure 17.   Results of proximate analysis of whole body constituents
             of juvenile fall chinook salmon collected during June 14-24
             (early), July 7-16 (mid), and August 2-11 (late), 1982.
             Fish were taken from the gatewells (GW) and just before they
             entered a raceway (RW) at NcNary Dam, and after transport to
             Bonneville Dam (PT).   All bars represent the means + SE for
             6 fish.
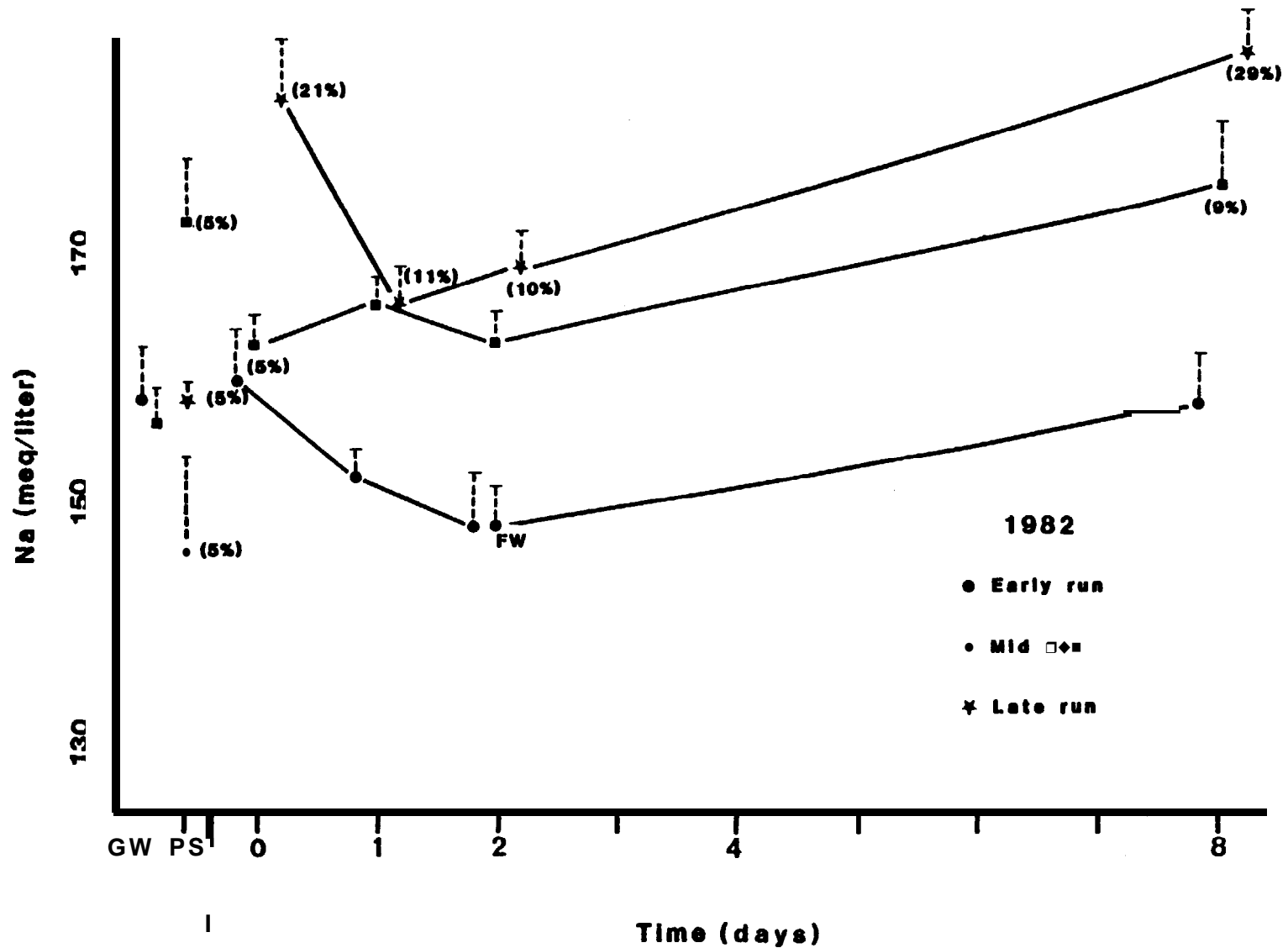
Figure 18. Plasma Na levels (mean + SE) of fall chinook salmon, 24 h after being put in 15 parts per thousand salt water or fresh water (FW). Fish were taken from the McNary Dam gatewell (GW) before passing the bar-sorter (PS) and during 8 d of recovery in the raceway after collection. Sampling was completed June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982. All points are duplicates of 10 fish each; percent mortality is in parentheses.
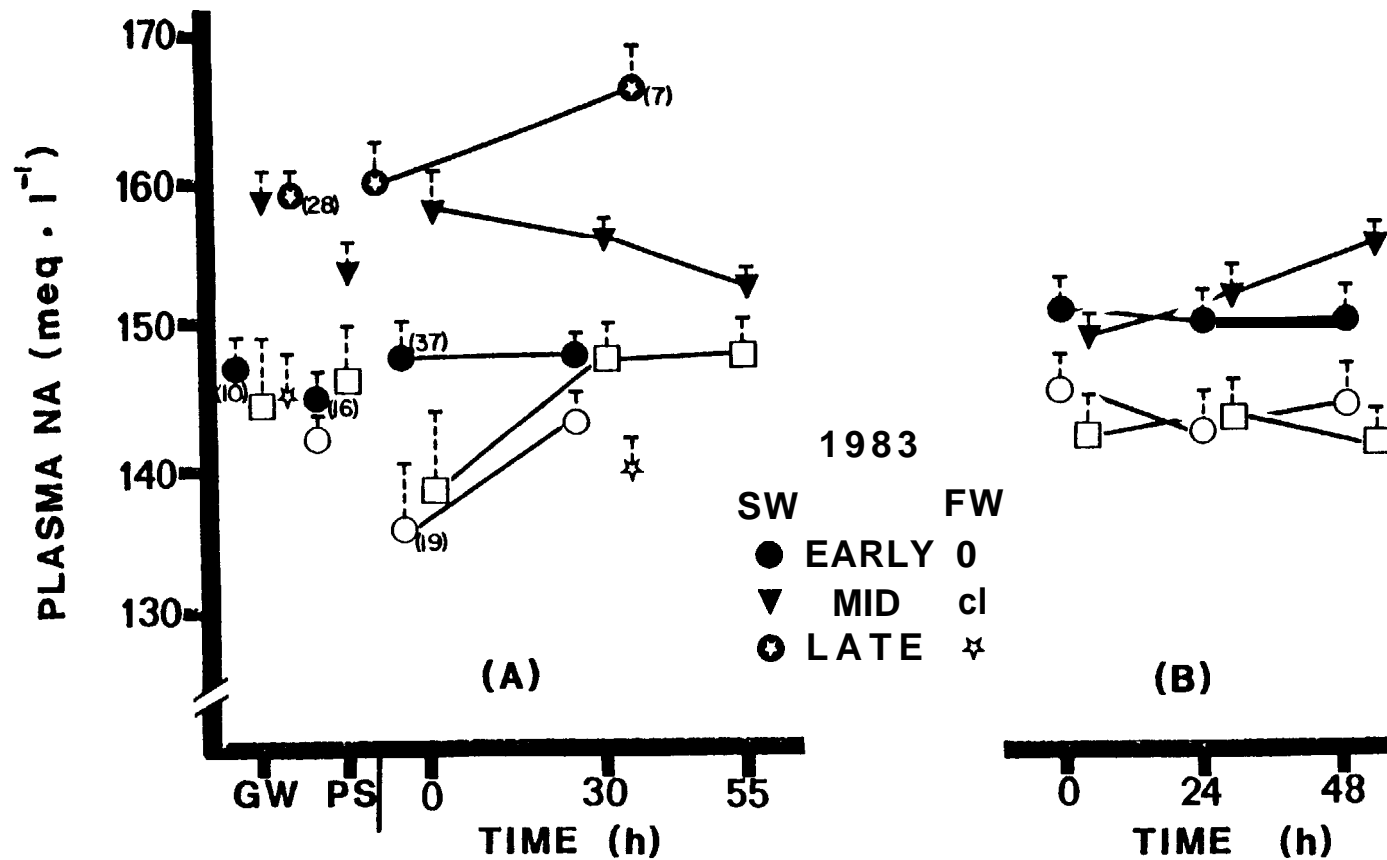
Figure 19. Plasma Na levels of outmigrant fall chinook salmon, 18 h after being put in 20 parts per thousand salt water (SW) or fresh water (FW). Fish were taken from McNary Dam gatewell (GW), just before the bar-sorter (PS) and after various recovery times in the raceway (A), and after various recovery times following transport to Bonneville Dam (B). All points are the mean + SE of replicate lo-fish groups challenged June 13-18 (early), July 7-16 (mid), and August 2-5 (late), 1983. Percent mortalities are in parentheses.
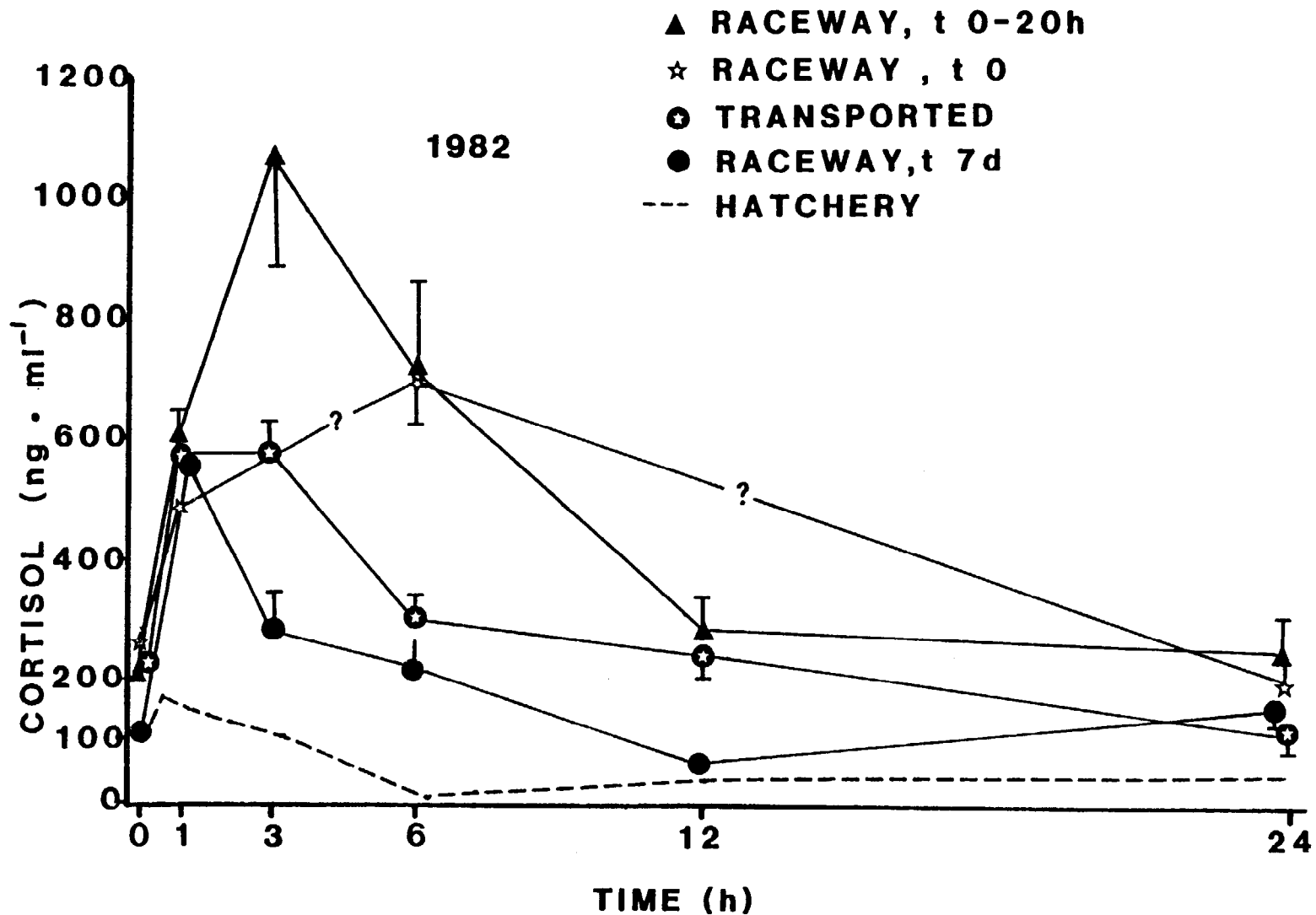
Figure 20. Plasma cortisol levels of outmigrating fall chinook salmon from McNary Dam, subjected to a 30-s handling stress by suspension out of water in a dipnet. Fish were taken from just before entry into a raceway, after 0 to 20 h, and 7-d raceway recovery, and after transport to Bonneville Dam. For comparison, results for hatchery, laboratory-acclimated fish subjected to the same 30-s handling stress are included. Points are mean + SE for 6-13 fish.

and fish transported to Bonneville had the same pre-stress plasma cortisol titers (CR. 200-250 ng*ml^{-l}), but the increase in cortisol of fish which had been in the raceway for a short time was twice that of transported fish (Fig. 20). This indicates that even though fish had comparable plasma cortisol levels, their response to another stress was affected by the length of time since the previous stress (see: Multiple stress). Cumulative effects of stress are also seen in fall chinook subjected to the secondary stress in 1983 (Fig. 21); however, the peak cortisol levels were considerably lower than in 1982 (ca. 375 vs. 1050 ng*ml-l). Plasma lactate was assayed for fall chinook which were secondarily stressed in 1982; however, there was no pattern of treatment effects (Fig. 22).

Spring chinook smolts were also stressed by the collection system as evidenced by their plasma cortisol responses (Figs. 23 and 24) which paralleled those of fall chinook sampled in 1983 (Figs. 4, 5, and 6). The spring chinook responded to the stresses of the collection system in a cumulative manner as shown by the increasing plasma cortisol levels in fish as they move through the system (Figs. 23 and 24). Cortisol levels of spring chinook also returned to baseline (i.e., < 100 ng*ml'l) within 24 h. Hepatic glycogen in spring chinook (Fig. 25) was consistently lower than levels in hatchery fish (fall chinook) fasted for 20 d, but did not reflect the continuing decrease which was seen with some fall chinook (Figs. 11 and 12). This is consistent with results for fasted fish (see: Nutrition and stress), and indicates that hepatic glycogen can be depressed only so far before other energy reserves are activated (i.e., lipids and muscle glycogen). Initial plasma glucose levels in spring chinook were significantly higher in early-run fish (ca. 75 mg*dl) than in late-run fish
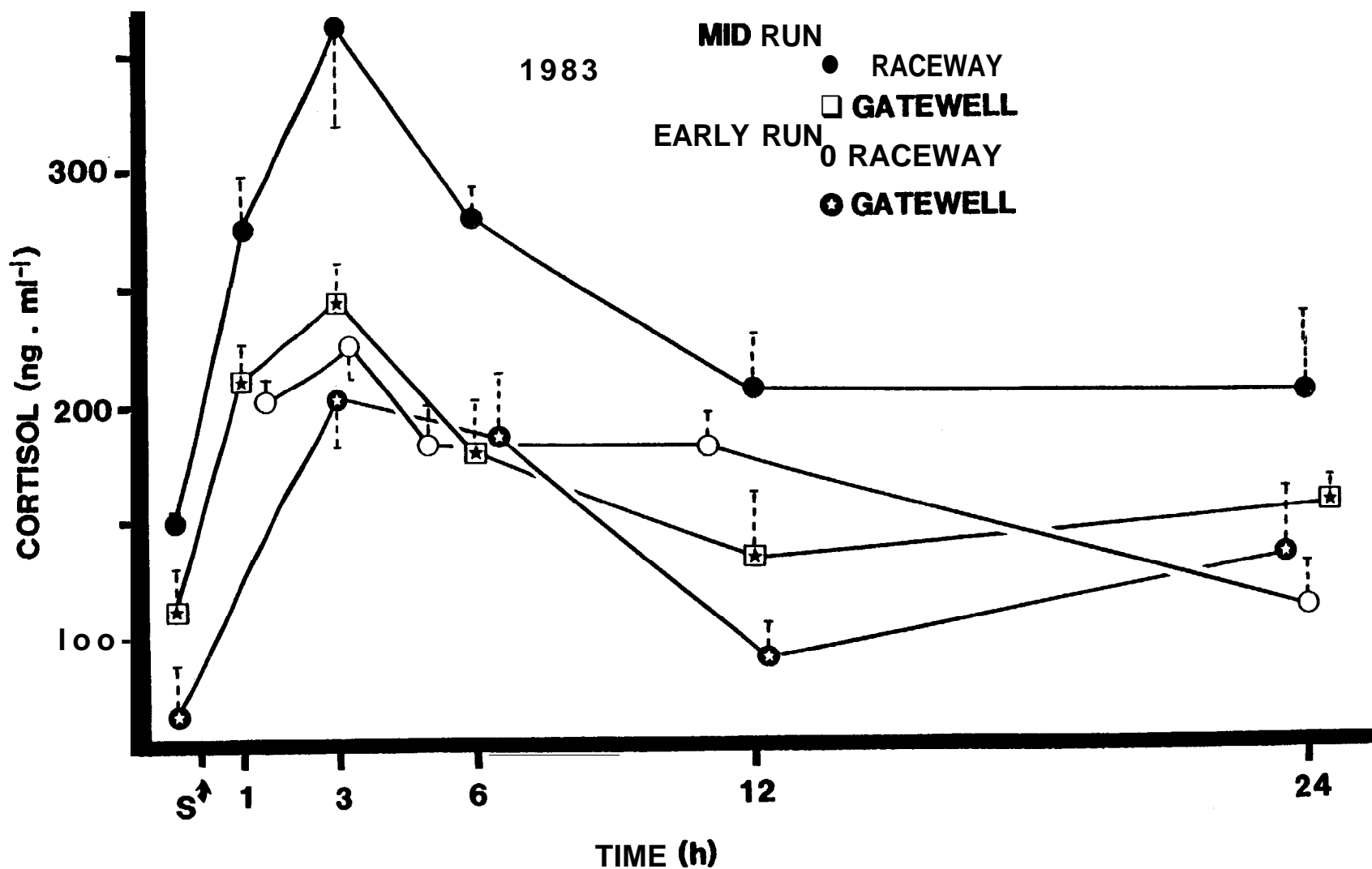
Figure 21. Plasma cortisol levels in fall chinook salmon collected from the gatewell or raceway-at McNary Dam, held in the air in a dipnet for 30 s, and released into a tank (ca. 100L) with flow-through water, and sampled through 24 h. Sampling was completed during June 14-19 (early run) and July 7-13 (mid run), 1983. All points are the mean + SE of 10 to 12 fish.
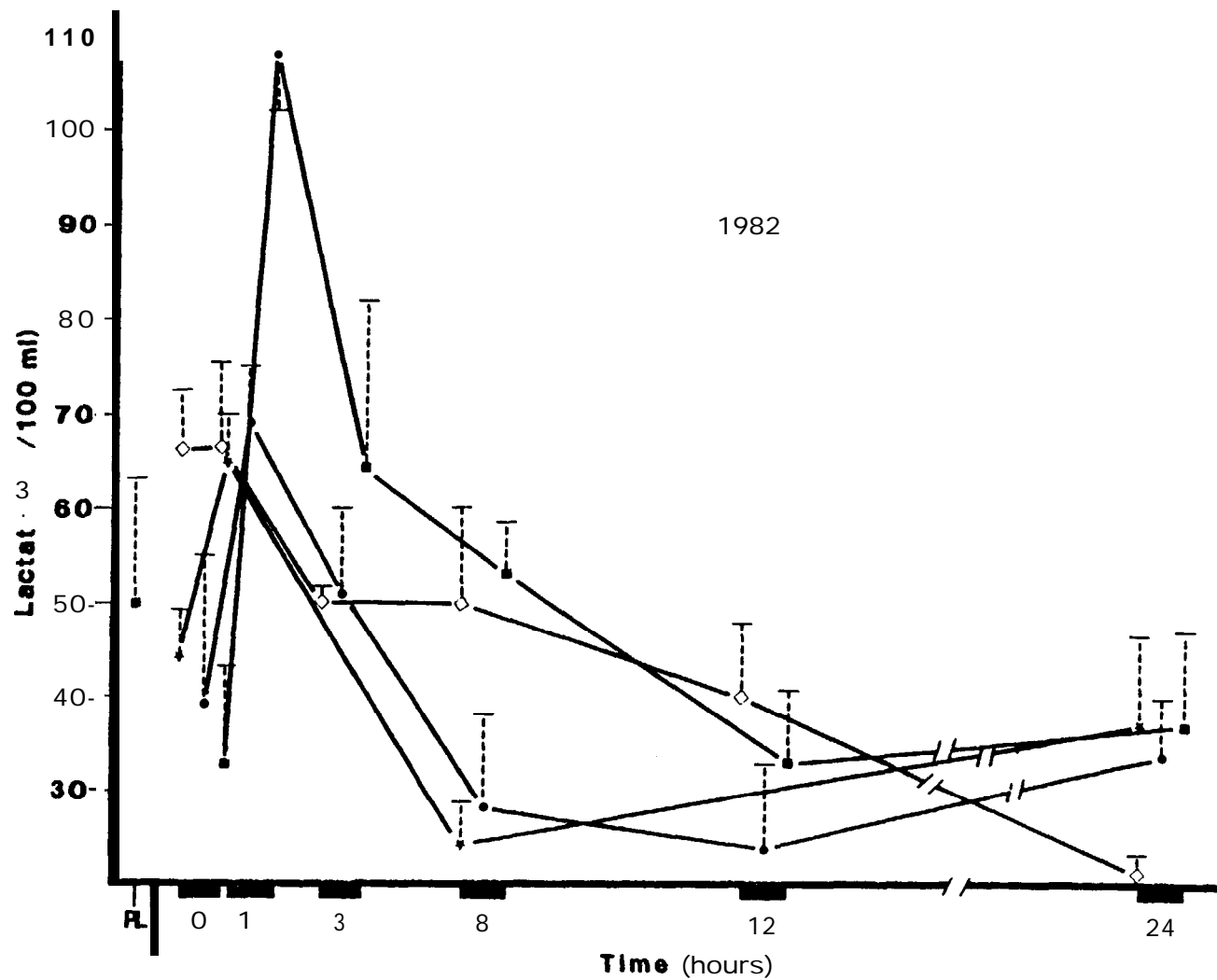
Figure 22. Plasma lactate levels (mean + 1 SE) of outmigrant fall chinook salmon subjected to a 30-s suspension out of water in a dipnet. Fish were taken from just before entry into a raceway (*, after 0-20 h (◇) and 7-d (●) recovery, and after being loaded into a truck (PL) and transported to Bonneville Dam (I). All points represent 3 fish.
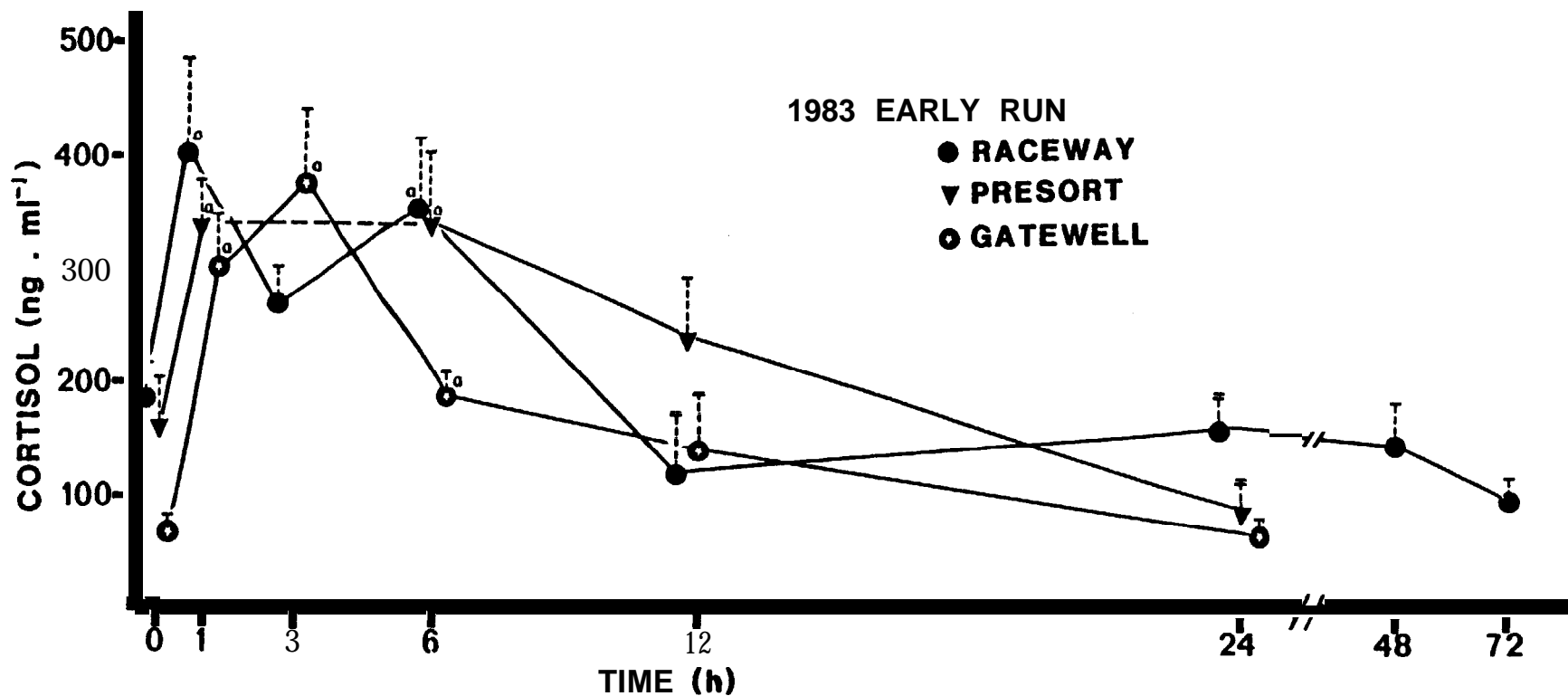
Figure 23. Plasma cortisol levels for outmigrating spring chinook salmon taken from the gatewell, just before the bar-sorter (presort) and the raceway, and after various recovery times in the raceway and in plastic buckets with flow-through water. Samples were collected at McNary Dam, May 3-6, 1983. All points are the mean + 1 SE of 10 to 12 fish. Points marked (a) are significantly different from Time = 0 of same line (P < .05, LSD test).
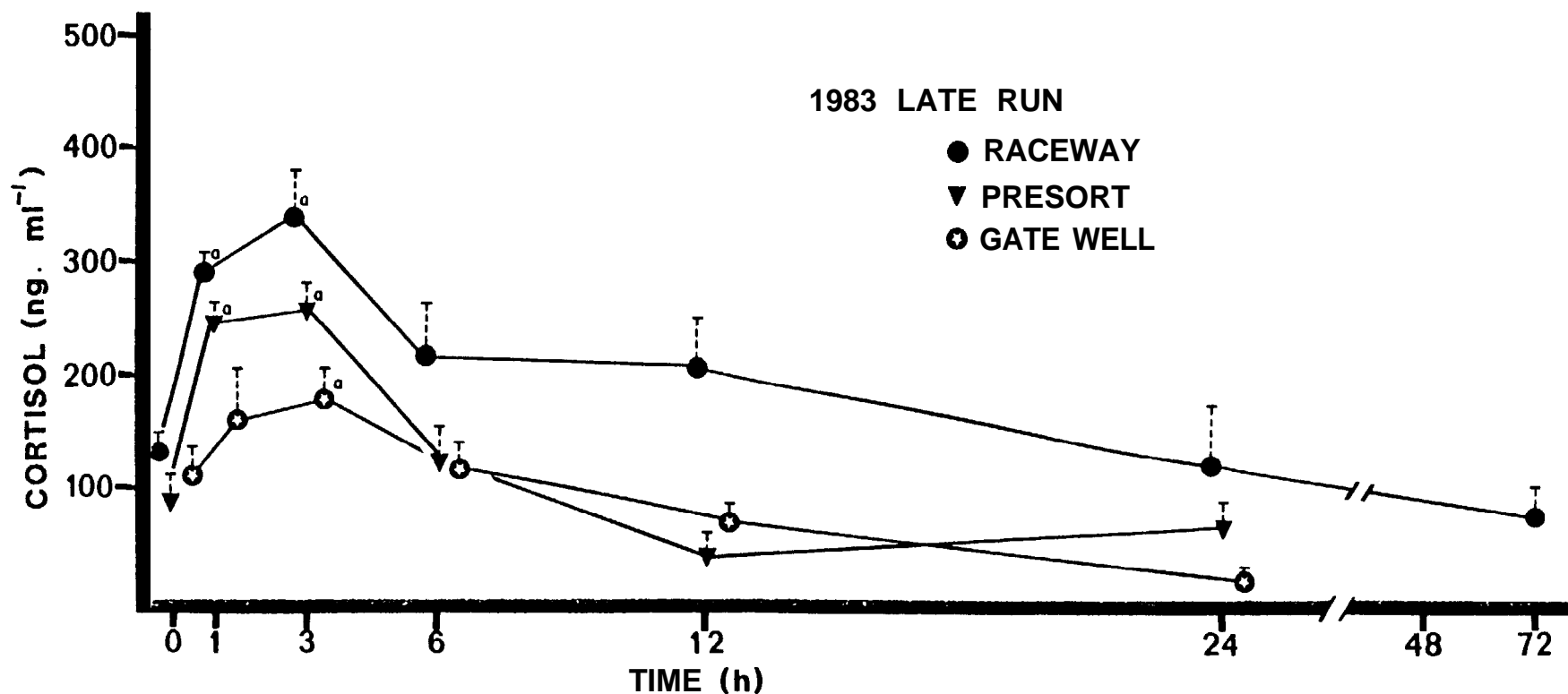
Figure 24. Plasma cortisol levels for outmigrating spring chinook salmon taken from the gatewell, just before the bar-sorter (presort) and the raceway, and after various recovery times in the raceway and in plastic buckets with flow-through water. Samples were collected at McNary Dam, May 23-27, 1983. All points are the mean +1 SE of 10 to 12 fish. Points marked (a) are significantly different from Time = 0 of same line (P < .05, LSD test).
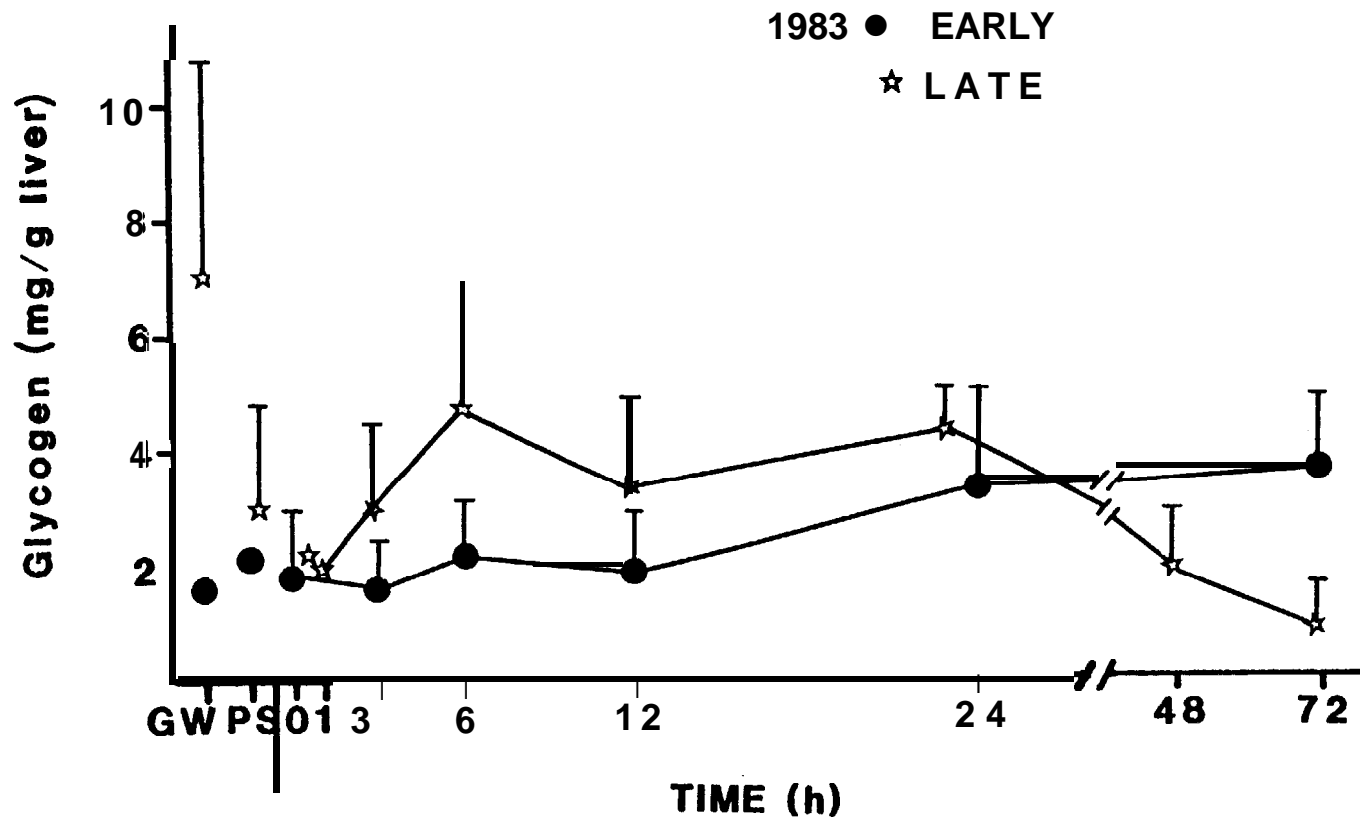
Figure 25. Hepatic glycogen levels in juvenile spring chinook salmon sampled from the McNary
Dam gatewell (GW), before passing the bar-sorter (PS) and during 72 h of recovery
in a raceway. Sampling was conducted during May 3-6 (early) and May 23-27 (late),
1.983. All points are mean + SE of 4 to 6 fish.

(ca. 35 $mg \cdot dl^{-1}$). These variations were probably the result of different histories (i.e., different origin and nutrition) of fish passing the dam at the different times (Figs. 26 and 27). The stresses of the collection system caused increases in plasma glucose to approximately the same level in early-and late-run spring chinook (Figs. 26 and 27), levels which were comparable to those of fasted fish subjected to a single 30-s stress (see: Nutrition and stress). Hematocrit and leucocrit values of spring chinook smolts showed considerable variability and no consistent patterns between runs (Fig. 28). WBC counts (Fig. 29) were also variable, but there appeared to be a depression in relative numbers of WBC between 0 to 48 h after the fish reach the raceway, similar to that found in fall chinook (Figs. 15 and 16).

Osmoregulatory capacity, as measured by saltwater challenges in spring chinook, was better in the late run than in the early run (Fig. 30), which may reflect the increased size of smolts (from 21.0 + 1.0 to 25.4 + 0.6 g) without a significant increase in water temperature (Fig. 9). Inexplicably, the osmoregulatory capacity of spring chinook taken from the gatewell (the sampling station we assumed was the least stressful) was reduced compared to fish from other parts of the collection system (Fig. 30). Response of spring chinook smolts to a secondary stress further illustrated the cumlative nature of stresses in the collection system (Fig. 31). Fish which were taken from the gatewell or after 1 h in the raceway had the same mean plasma cortisol levels (ca. 150 $ng \cdot ml^{-1}$). However, the plasma cortisol of fish from the gatewell peaked at approximately 275 $ng \cdot ml^{-1}$, while levels in fish from the raceway peaked at approximately 410 $ng \cdot ml^{-1}$. There was a difference between fall and spring chinook in their response to the
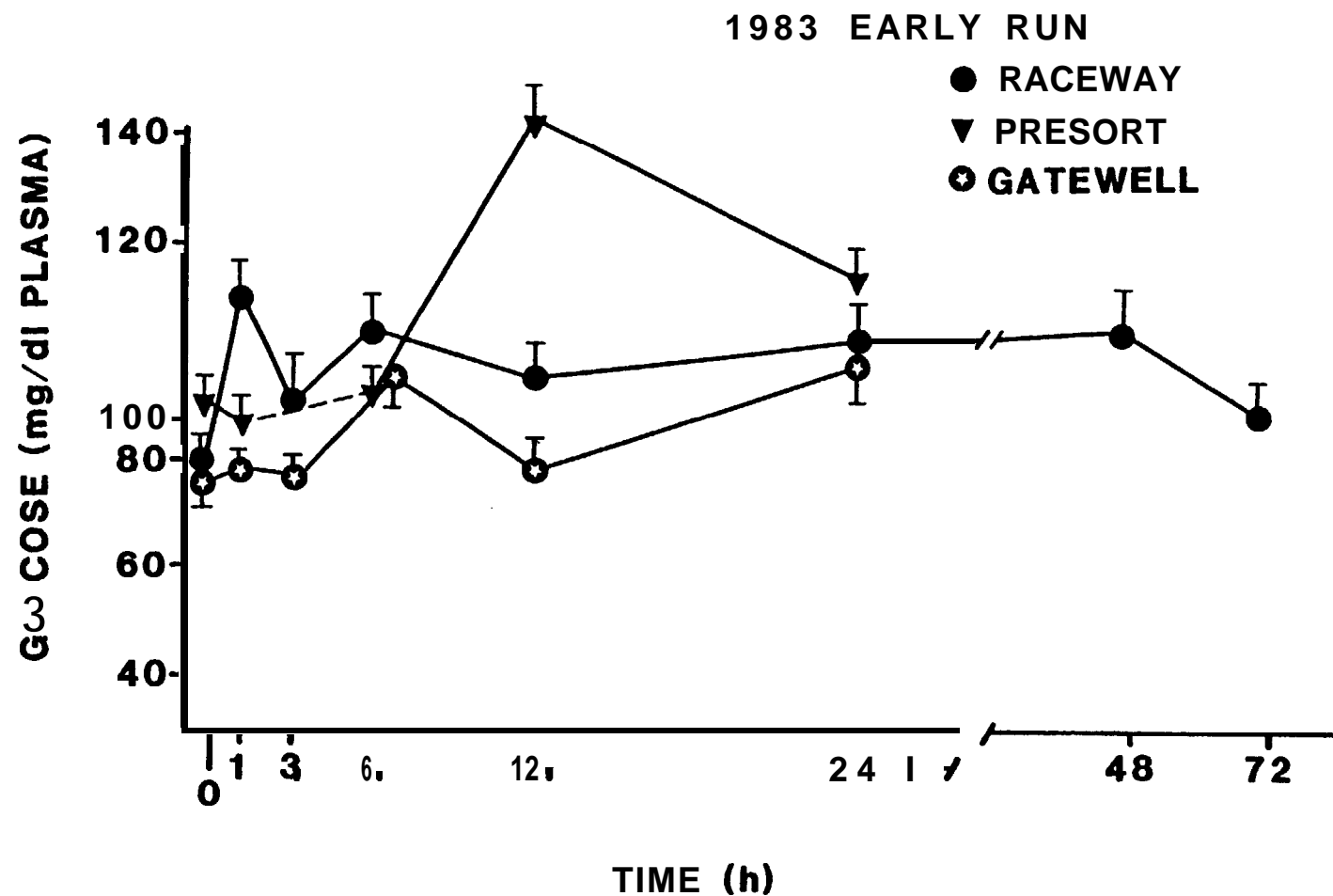
Figure 26. Plasma glucose in juvenile spring chinook salmon taken from the McNary Dam gatewell, just before the bar-sorter, and the raceway; and after various recovery times in the raceway and in plastic tanks with flow-through water. Points are mean + SE of 10 to 12 fish collected during May 3-6, 1983.

1983 LATE RUN
- ● RACEWAY
- ▼ PRESORT
- ⊘ GATEWELL
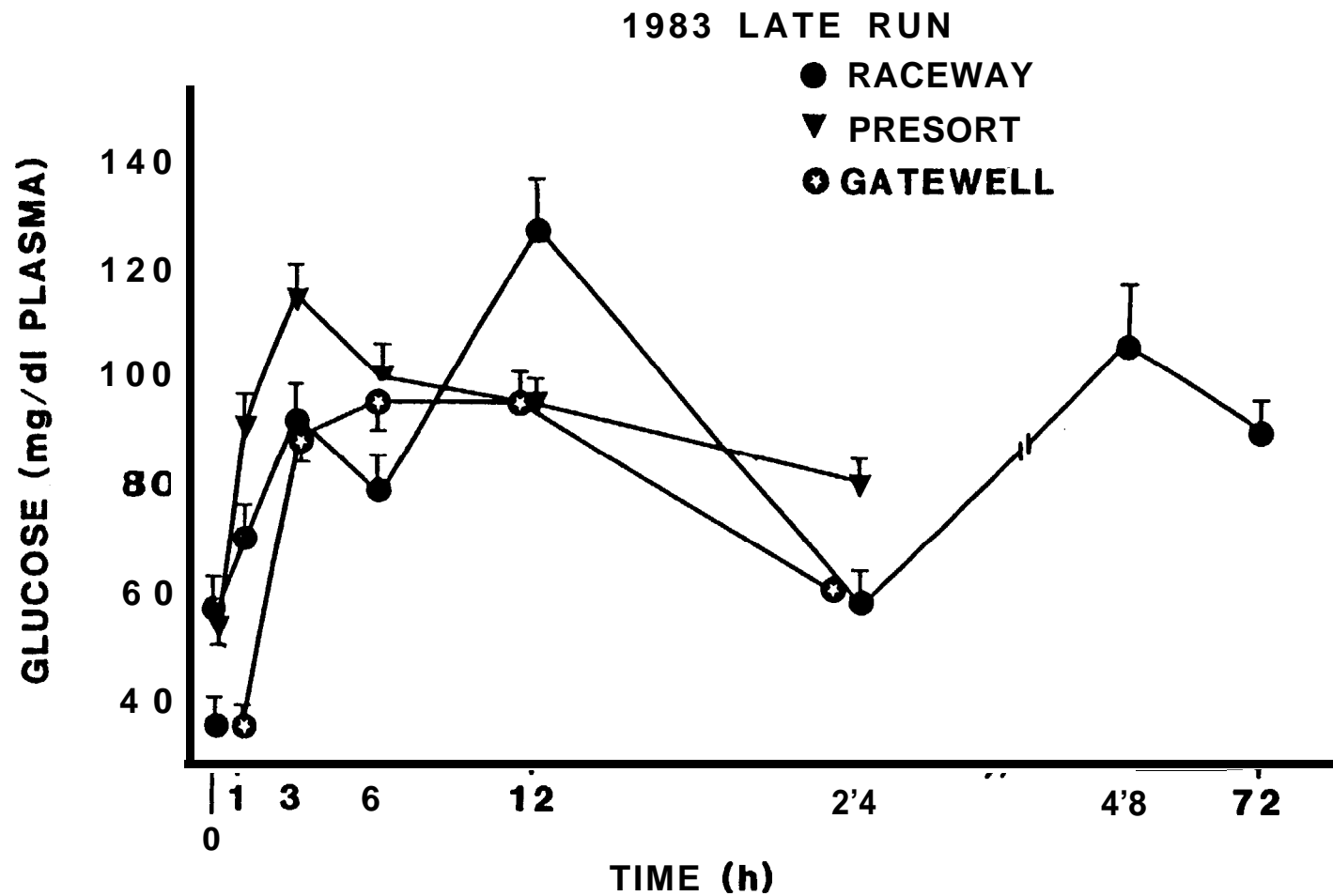
Figure 27. Plasma glucose in juvenile spring chinook salmon taken from the McNary Dam gatewell, just before the bar-sorter, and the raceway; and fish sampled after various recovery times in the raceway and in plastic tanks with flow-through water. Points are mean + SE of 10 to 12 fish collected during May 23-27, 1983.
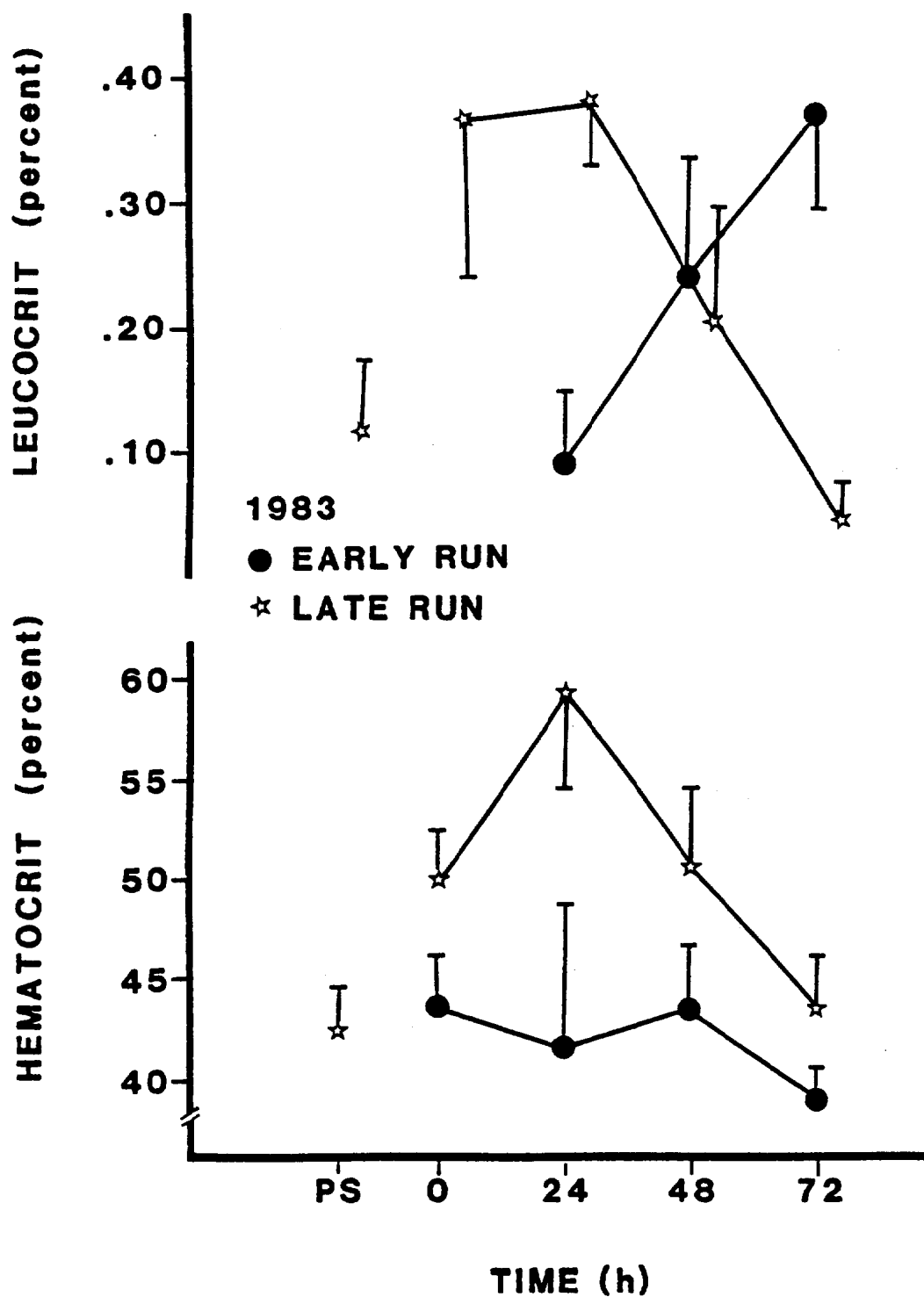
Figure 28. Leucocrit and hematocrit values in juvenile spring chinook salmon collected from McNary Dam during May 3-6 (early run) and May 23-27 (late run), 1983. Fish were sampled just before the bar-sorter (PS) and through 72 h of recovery in a raceway. Points are the means + SE of 6 fish.
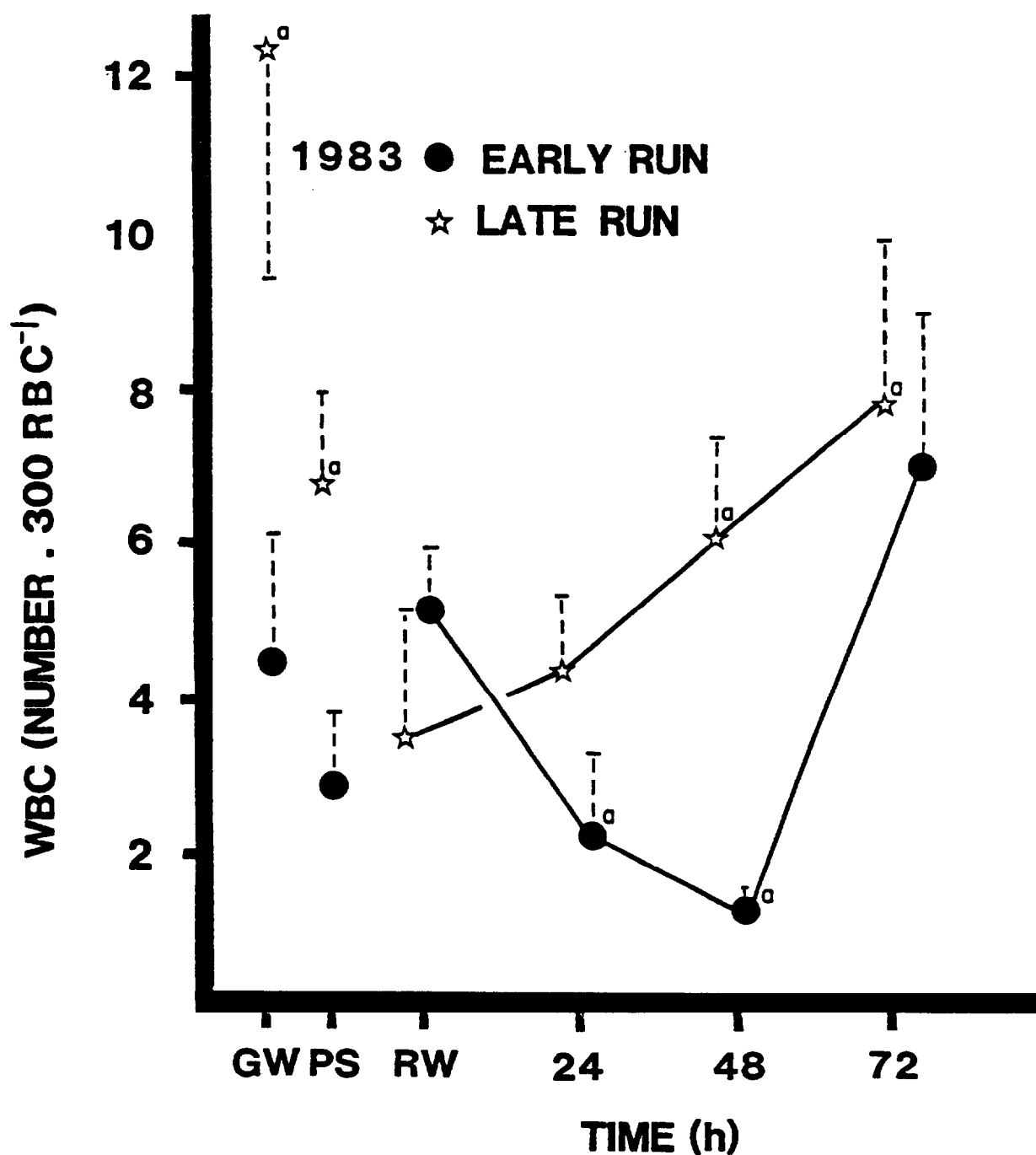
Figure 29. White blood cell (WBC) counts for outmigrating spring chinook salmon
collected from the gatewell (GW), just before the bar-sorter (PS),
just before entering the raceway (RW), and after various recovery
times in the raceway. Samples were collected during May 3-6 (early
run) and May 23-27 (late run), 1983. All points are the means + SE
of the average of two replicate counts of the number of WBC's among
300 erythrocyte (RBC) on'the blood smears from 6 fish. Points
marked (a) are significantly different from RW of same line
(P < .05, LSD test).
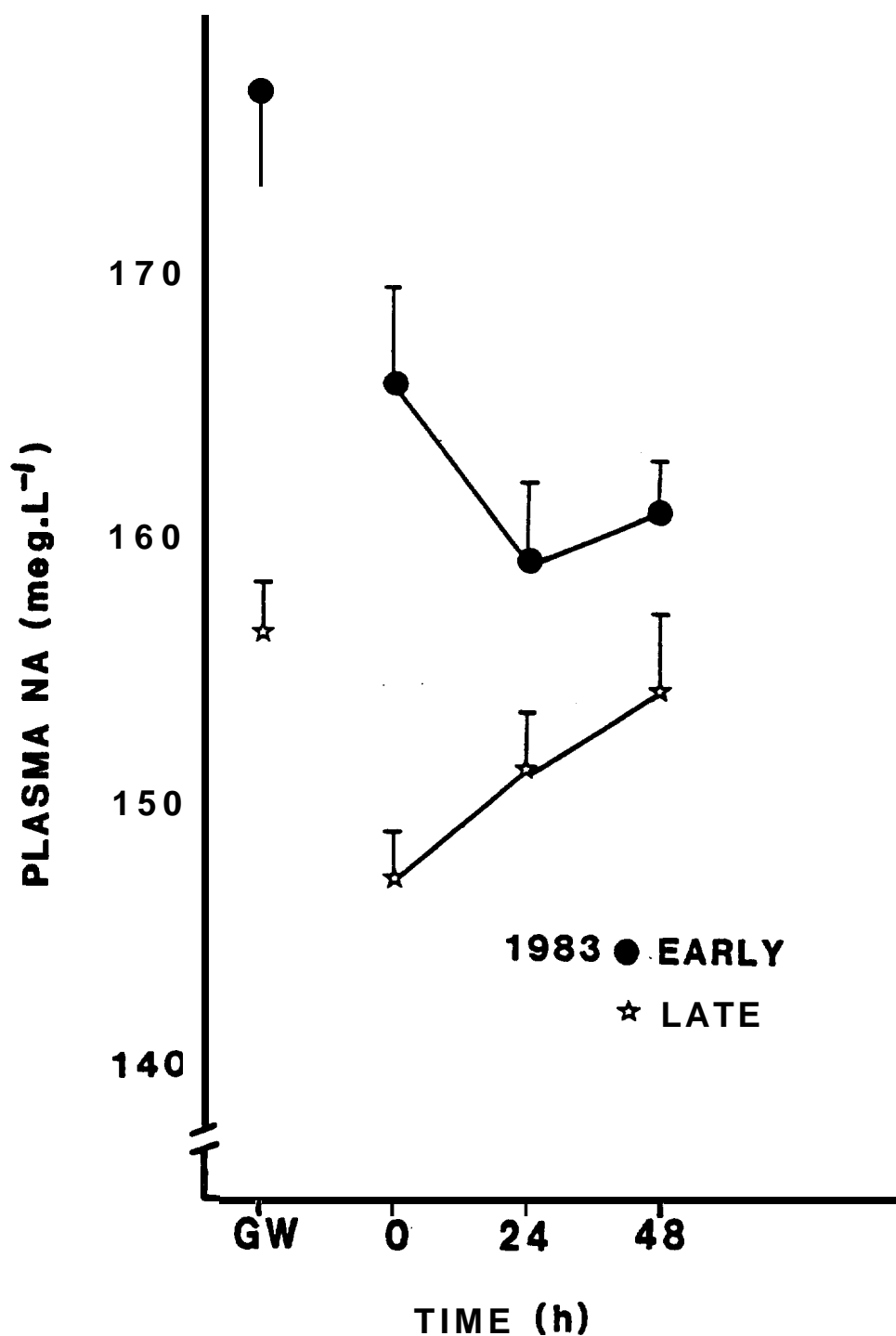
Figure 30. Plasma Na levels in juvenile spring chinook salmon, 18 h
            after being put in 17 parts per thousand salt water.   Fish
            were taken from McNary Dam gatewell (GW) after various
            recovery times in the raceway.   All points are the mean + SE
            of replicate lo-fish groups challenged May 3-6 (early) and
            May 23-27 (late), 1983.
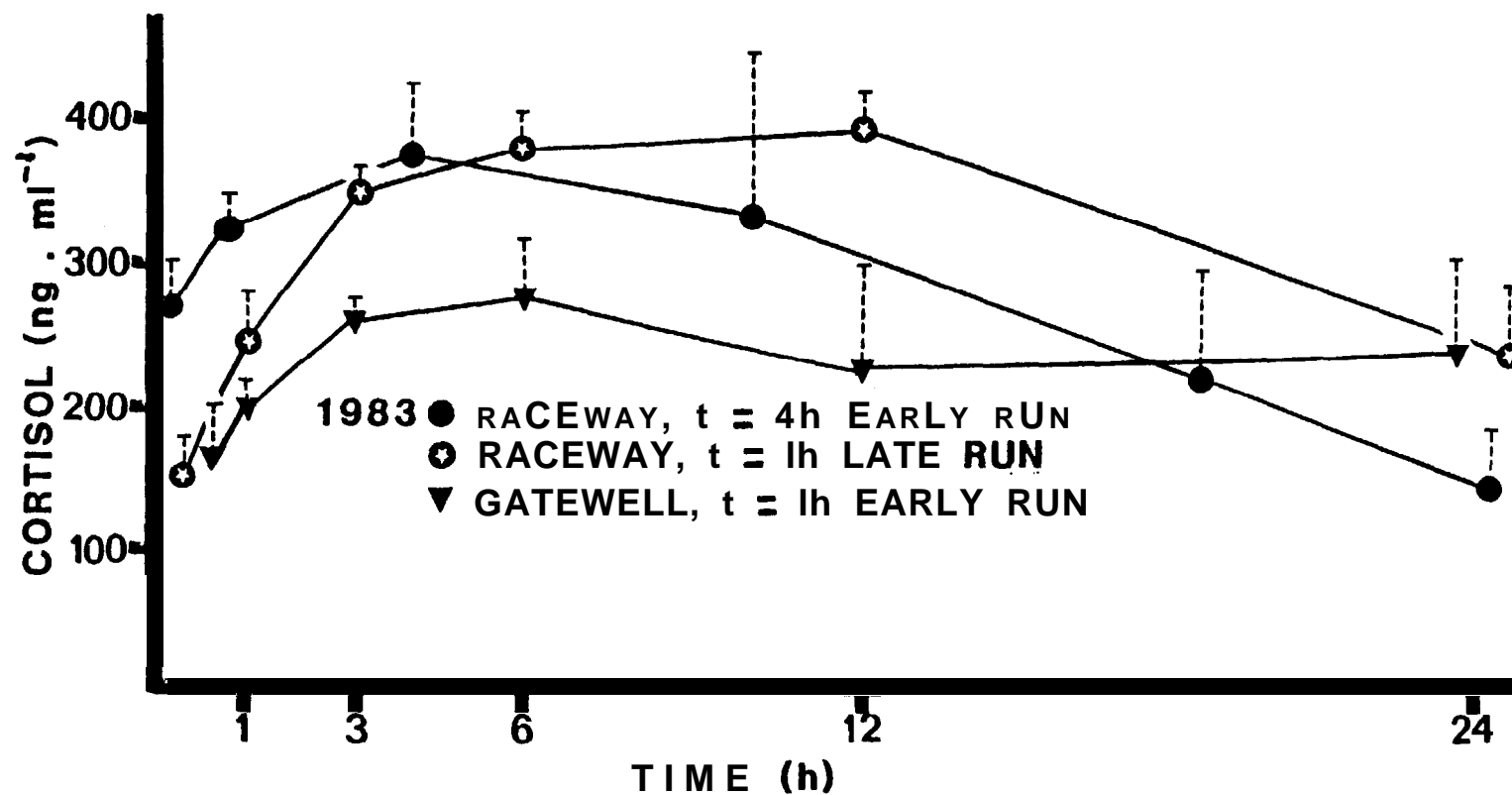
Figure 31. Plasma cortisol levels for outmigrating spring chinook salmon taken from the gatewell and the raceway, and suspended in the air in a dipnet for 30 s and allowed to recover in plastic buckets with flow-through water. Samples were collected at McNary Dam, May 3-5 (early) and 23-27 (late), 1983. All points are means + SE of 10 to 12 fish.

secondary stress. Plasma cortisol levels in fall chinook were generally reduced 12 h after the secondary stress (Figs. 21 and 22) while cortisol response in spring chinook remained elevated beyond 12 h (Fig. 31).

Raceway density evaluation. Loading raceways with fall chinook to the established maximum of 0.5 $lbs \cdot gal^{-1}$ did not appear to have serious long-term effects on the fish, as judged by plasma cortisol levels (Fig. 32). It appeared that increasing density from 0.13 to 0.25 to 0.5 $lbs \cdot gal^{-1}$ caused an increase in initial plasma cortisol levels, but most cortisol levels were significantly reduced after 24 h. The initial plasma cortisol response of fish held in the raceway at 0.5 $lbs \cdot gal^{-1}$ was highest in fish sampled in the late run followed by the mid-run and early run during 1982 (Fig. 32), indicating that there may have been some environmental factor or predisposing factor in the fish which affected the cortisol response to stress as the run progressed. During both the mid-run and late run, 1983, plasma cortisol levels were approximately 150 $ng \cdot ml^{-1}$ in fall chinook smolts as they entered the raceway, and approximately 100 $ng \cdot ml^{-1}$ after the fish were held at 0.5 $lbs \cdot gal^{-1}$ for 24 h (Figs. 5 and 6), suggesting that there was no difference in the response to maximum loading density through the 1983 run. Sampling techniques were different between 1982 and 1983 and so the data are not comparable between years. Spring chinook smolts were held at 0.5 $lb \cdot gal^{-1}$ during both sampling periods in 1983. During the early portion of the run, smolts entering the raceway had plasma cortisol levels of approximately 190 $ng \cdot ml^{-1}$ as compared to approximately 160 $ng \cdot ml^{-1}$ during the late run (Figs. 23 and 24). After 24 h in the raceway, cortisol levels were approximately 140 and 130 $ng \cdot ml^{-1}$ in early-run and late-run fish, respectively (Figs. 23 and 24).
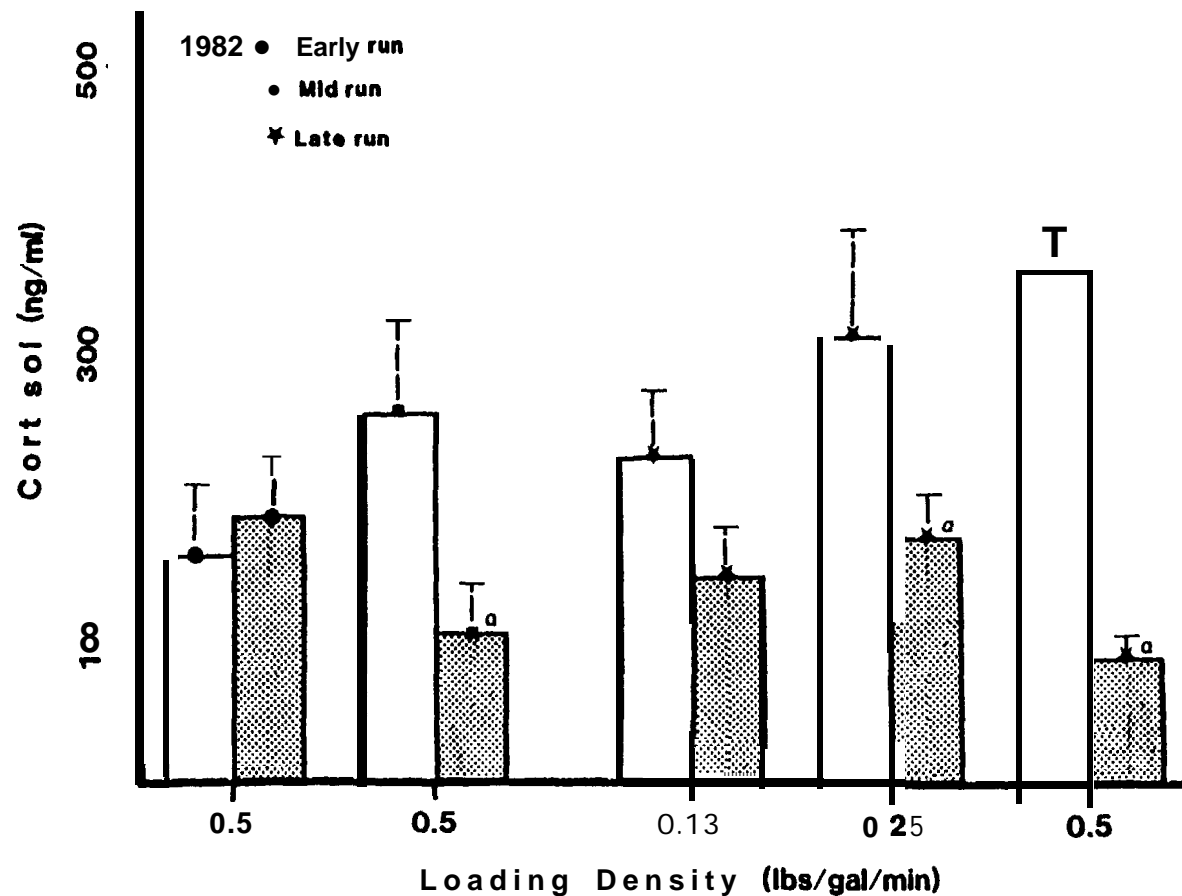
Figure 32. Plasma cortisol levels of juvenile fall chinook salmon collected at McNary Dam and allowed to recover in raceways at various loading densities. Samples were taken after raceways were loaded and fish crowded to appropriate density (clear bars) and 24 h after crowding (stippled bars). Points are means + SE for n = 11 to 13. Points marked (a) are significantly different from values of fish immediately after they were crowded to appropriate density (P < .05, LSD test).

The elevated initial plasma cortisol levels in fall chinook sampled during 1982 are undoubtedly caused by the sampling procedure which required crowding the fish into one end of the raceway prior to taking the sample of fish. However, the important point is that in almost all instances, mean plasma cortisol levels were reduced after 24 h regardless of the loading density. Thus, there appears to be no advantage in holding smolts at less than 0.5 $lbs \cdot gal^{-1}$ prior to transport.

Anesthetic, handling, and marking. Plasma cortisol values were significantly higher in fish sampled from the marking facility at McNary Dam in 1982 as compared to 1983 (Fig. 33). After initial examination of the 1982 results, we speculated that the increase in plasma cortisol between 24 and 48 h might have been the result of an inflammatory or other response to the presence of the CWT in the fish's snout. The 1983 results failed to confirm this and, in fact, it appeared fish with a CWT were no more stressed than fish which were only branded. Moreover, within 12 h, fall chinook plasma cortisol had declined to pre-mark levels and remained low for at least 72 h. It appears that marked fish were not stressed more than fish which went through the collection system without being handled, but that at least 12 h was required for recovery. This does not address the response of smolts to additional stresses of being loaded into a transport vehicle and released into the river. That is, we do not know if these marked fish are any more or less susceptible to additional stress than non-marked fish (see: Multiple stress).
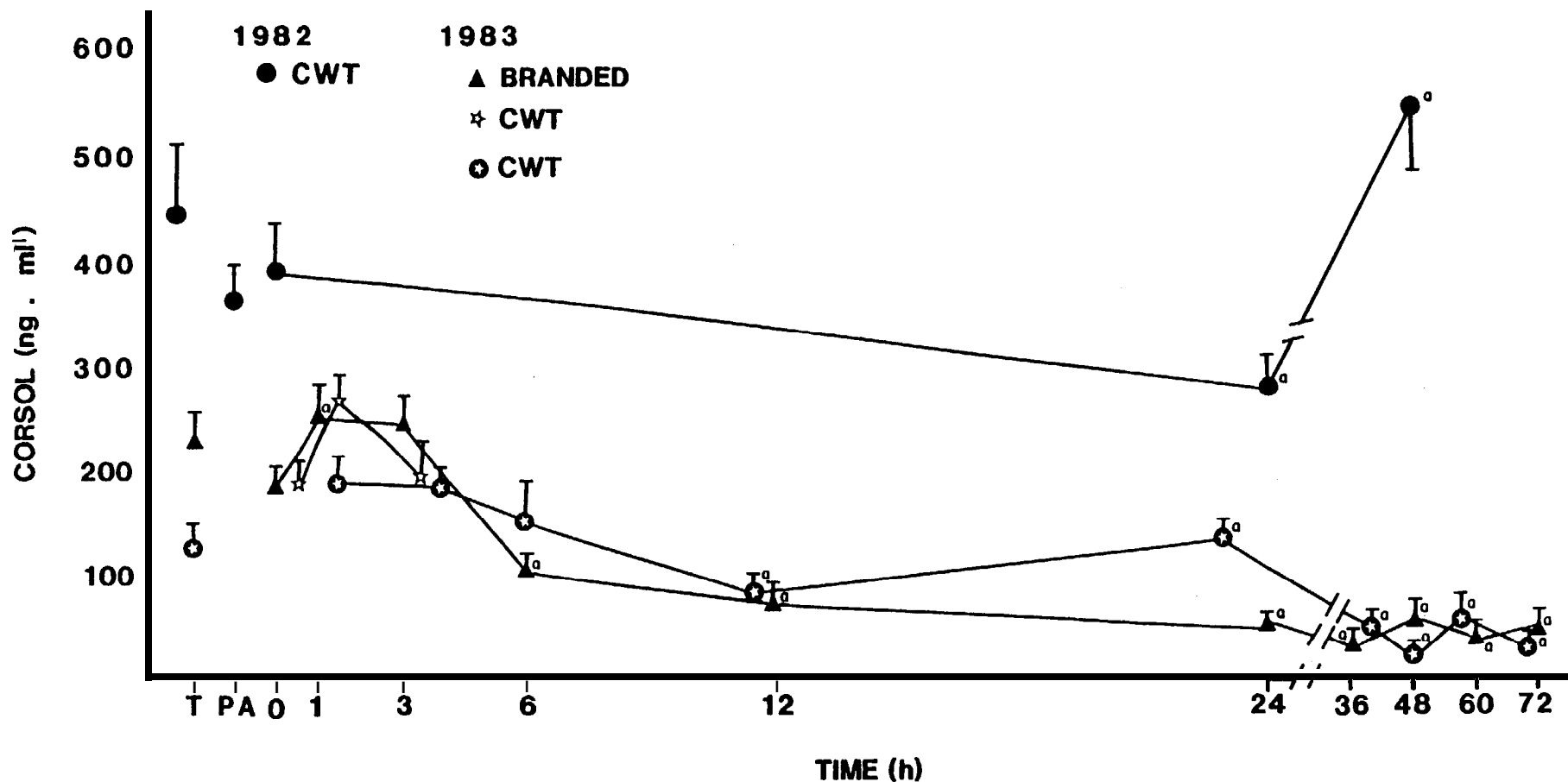
Figure 33. Plasma cortisol levels in juvenile fall chinook salmon collected from the marking facility at McNary Dam during July 14-22, 1982, and July 7-13, 1983. Fish were sampled from the holding tank (T), and after they were anesthetized (PA), and after they were fin-clipped and cold-branded (BRANDED), and after they were branded and had coded wire tags inserted into their snouts (CWT). Branded and CWT fish were also collected, held in large plastic tanks (ca. 100L) with flow-through water, and serially sampled through 72 h. All points are the means + SE of 10 to 12 fish. Points marked (a) are significantly different from T = 0 of same line (P < . 05, LSD test).

TRANSPORT EVALUATION

Experimental Rationale and Methods

Truck transport. The U.S. Army Corps of Engineers maintains a fleet of fish transportation tank trucks. The tanks had a maximum capacity of 3,500 gal (13,250 L) and could be divided into three compartments of 25, 25, and 50% of the total volume. Water temperature was controlled by a refrigeration unit, and 5-6 $l\cdot min^{-1}$ of bottled oxygen was bubbled through the water, a rate which previously had been determined to maintain oxygen at or near saturation (Brad Eby, U.S. Army Corps of Engineers, personal communication). Fish which had been in the raceways for a maximum of 48 h, were loaded into the tanks by crowding them to one end of the raceway into a sluiceway which emptied Into the tank, a drop of approximately 1 to 2 m. After the 3- to 4-h trip to Bonneville Dam, the fish were released back to the river via a port at the rear of the tank. In 1982, fish were flushed out of this port and into a long tube (ca. 100 x 0.5 m) which emptied at the water surface downstream of the Bonneville Dam first powerhouse. In 1983, truck-transported fish were released directly into the river at Dalton Point boat ramp, approximately 21 km below Bonneville Dam.

In order to examine the effects of the transportation procedures (Objective 2a) and to determine the optimal, if any, recovery time for transported fall chinook smolts (Objective lb), we collected fish after truck transport to Bonneville Dam during June 16-24, July 14-22, and August 2-10, 1982; and June 15-18 and August 16-18, 1983. For this evaluation, we collected fish which had been in a raceway for 48 h, fish which had just been loaded into the truck, and fish immediately after

transport to Bonneville Dam. We also removed fish from the trucks at
Bonneville Dam, held them in 1.2 m circular fiberglass tanks with
flow-through river water, and monitored recovery rates through 8 d (1982).
In 1983, fish were held in large, dark plastic tanks and were serially
sampled through 72 h.

   Disease challenge of transported fish. Fall chinook salmon were
transported from the raceways at McNary Dam to the Oregon State University
Marine Science Center, Newport, Oregon, on June 17, July 8, and August 9,
1982. For each transport run, about 500 fish were netted from the raceway
in the early morning and carried in water buckets to the transport truck.
Each transport run lasted 8 to 9 h (distance about 645 km). During this
time, aeration was supplied by a water recirculation pump. Water
temperature was maintained at ambient river temperature (+ 1.0 C) by adding
non-chlorinated ice, as needed. At the Marine Science Center, 300 fish were
equally distributed among twelve 0.61-m circular tanks. Six of the tanks
held fresh water throughout the experiment and the other six were changed to
saltwater when all tanks were inoculated with 8.5 ml of Vibrio anguillarum
serotype I in trypticase soy broth. The final concentrations of Vibrio were
1.4 to 2.0 x 10^5 cells per 1.0 ml of aquarium water as determined by plate
count. In an effort to determine post-transport recovery rates, we exposed
fish in replicate saltwater and freshwater tanks to Vibrio within L h of
arrival, and two replicate sets of freshwater and saltwater tanks were
exposed 1 d after transport and 8 d after transport. Exposure was
accomplished by shutting off the water supply for 30 minutes and pipetting
the prepared Vibrio culture into each tank. The number of mortalities in
each tank and the presence or absence of brands on the mortalities were

noted daily from the beginning of the experiment until 13 d after exposure to Vibrio.

The percent mortality **and mean of the times to death (MTD)** were calculated for each tank with the branded and transported fish considered separately.

$$\text{MTD} = \frac{\text{(Mortalities/day) (days from challenge)}}{\text{total mortalities during challenge}}$$

$$\text{Percent mortality} = \frac{\text{number dead during challenge}}{\textbf{total number} \text{ of fish at start of challenge}}$$

Fish that died before exposure to Vibrio were not included in the calculations, When fish in two tanks experienced equal mortality, but the MTD of fish in one was significantly higher than in the second, we concluded that fish in this second tank were better able to resist the lethal effects of the **disease**.

About 2,000 juvenile fall chinook from Priest Rapids Hatchery, Washington, were transported to the Marine Science Center on May 28, 1982, in a 757-liter tank with a water recirculation system for aeration. During the 8-h trip, water temperature was maintained at 13.0 + 1.0 C with non-chlorinated block ice. The fish were placed in two 0.91-m circular, freshwater holding tanks to serve as controls for disease challenge and growth experiments. Six to 10 d before the June and July transportations of fish from McNary Dam to the Marine Science Center, 360 fish were cold-branded and equally distributed in twelve 0.61-m circular, freshwater flow-through tanks for use in the disease challenges. Apparently these fish

were in poor condition when brought from the hatchery, as between June 16 and July 21, a protozoan disease (<u>Ichthyophthirius</u> sp.) resulted in almost total mortality. Although these fish were nominally used as controls in the disease challenges, their initial poor health negated their value. We therefore did not include the results from this portion of disease challenge to avoid confounding the overall experimental results.

<u>Seawater growth and survival</u>. Growth and survival studies were conducted on fall chinook salmon transported from McNary Dam to the Marine Science Center in the same truck as that used to transport fish for the <u>Vibrio</u> challenge (June 17, July 8, August 9, 1982). Upon arrival at the Marine Science Center, the fish were netted from the truck and placed in two 0.91-m circular seawater tanks. The fish were fed Oregon Moist Pellets daily to satiation. Dead fish were removed and recorded daily for a 15-d period.

We did not want the stress of anesthetization and handling to interfere with this growth experiment, so the initial weights and lengths were not taken from the actual fish used in the experiments. For the June experiment, we used the mean weights and lengths of fish collected at McNary Dam (n = 76) and in July and August initial weights and lengths were determined from a subsample of fish (n = 30) taken from the transport tank but not used in the growth experiment. The mean daily minimum and maximum water temperatures during the study were 12.0 and 16.0 C, respectively, in June and August, and 13.5 and 17.2 C, respectively, in July. Salinity was 32.5 + 0.5 parts per thousand throughout the studies.

At the end of the 15-day growth period, fish from the experimental growth tanks were anesthetized, measured, and weighed. In August, the weighed fish were returned to their tanks, and after another 15 d, were reweighed and measured. In late July we obtained fall chinook from Trask Fish Hatchery, Tillamook, Oregon, as controls for the August seawater growth experiment.

Transported fish density. The environmental conditions during transportation of fish can have an effect on physiological indices of stress and on survival (Specker and Schreck 1980; Barton and Peter 1982). One aspect of this environment is the density of fish during transport. To investigate whether or not transport densities had differential effects on fall chinook smolts (Objective 4c), we: 1) evaluated data obtained from the regular transport runs which was monitored in 1982 and 1983; 2) during August 8-9, 1982, we compartmentalized an Army Corps of Engineers truck and loaded the compartments with fish to either 0.1, 0.5, or 0.8 $lb \cdot gal^{-1}$ (0.01, 0.06, or 0.10 $kg \cdot L^{-1}$); 3) during August 5-10, 1982, we used a small pickup truck-mounted transport tank to simulate the large truck transport. The capacity of the small tank was 200 gal (757 L), water temperature was controlled by a refrigeration unit, and oxygen was maintained at 11 + 1 parts per million by bottled oxygen. In all transport trials, fish which had been in a raceway for a maximum of 48 h were sampled after they were loaded into a transport vehicle, and again after a 3- to 4-h transport.

Barge transport.   During the major portion of the 1983 fall chinook
emigration, collected fish were transported by barge.   During July 14-22,
1983, we investigated the effects of barge transport on fall chinook smolts.
Fish were collected before and after being loaded into a barge, during the
barge transport trip, and upon arrival at Bonneville Dam.   Fish were also
removed from the barge at Bonneville and held in dark, plastic tanks with
flow-through water and serially sampled through 72 h.

Results and Discussion

Truck transport.   The overall transportation protocol was stressful to fall
chinook smolts.   However, the fish were stressed during the processes of
loading and, perhaps, unloading rather than from the transport vehicle.
There were two trends clearly indicated in the 1982 (Fig. 34) and 1983
(Figs. 35 and 36) plasma cortisol results.   First, plasma cortisol levels
increased between fish in the raceway and immediately after they were loaded
into a truck (Figs. 34 and 35) or barge (Fig. 36). Second, there was a net
decrease in plasma cortisol levels between the time fish were loaded into a
truck (Figs. 34 and 35; also see Fig. 44) or barge (Fig. 36), and after
3 to 4 h in transit to Bonneville Dam.   There were no significant
differences in plasma cortisol levels of fall chinook removed from the truck
at Bonneville Dam at different times of the run in 1982 (Fig. 34) or 1983
(Fig. 35).   The highest plasma cortisol levels were comparable between
years, but the 1982 data indicated that levels peaked 1 d after transport.
The 1983 plasma cortisol levels reached a maximum within 3 h and were
reduced 24 h after fish were removed from the truck.   We believe that
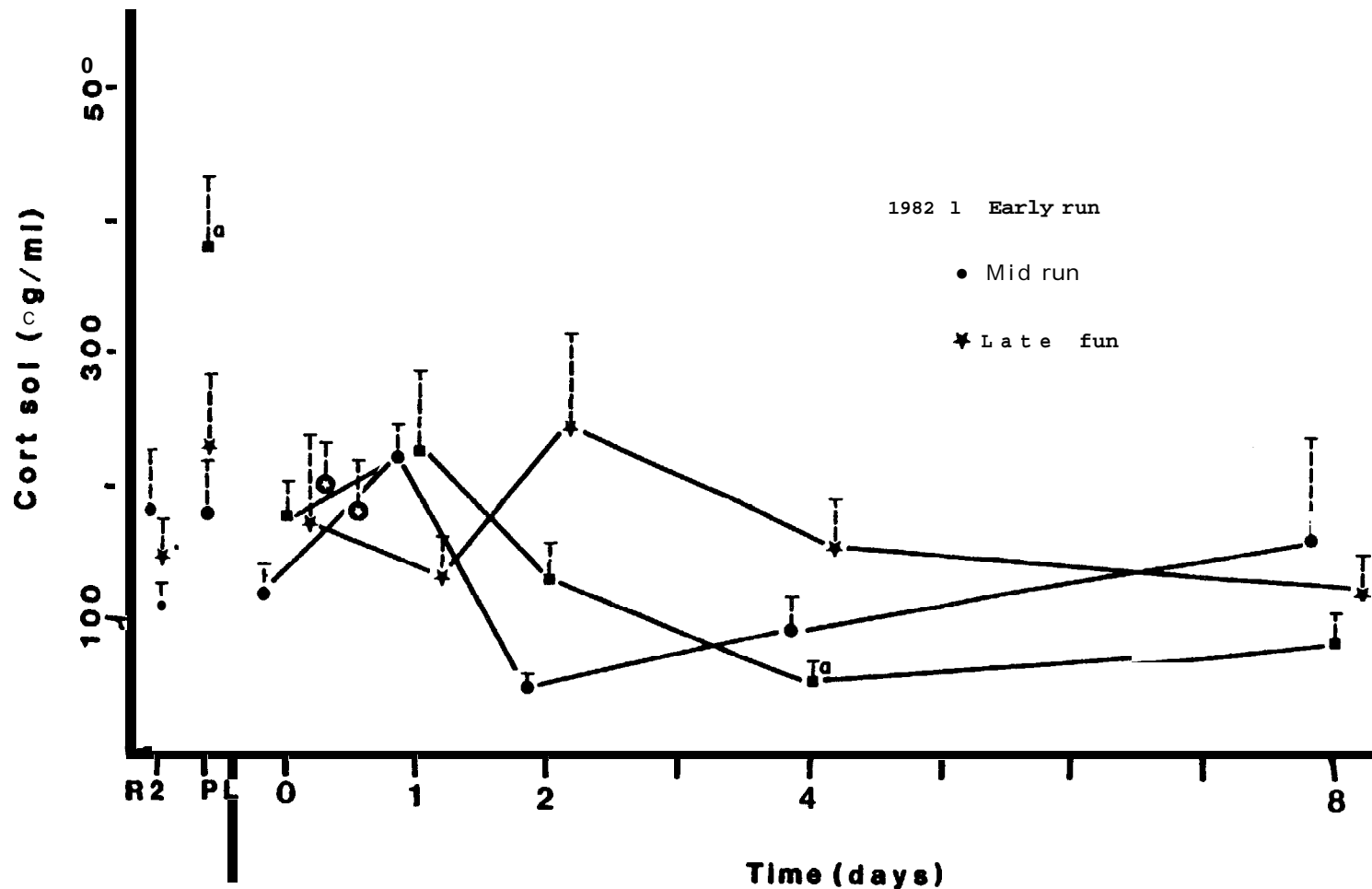
Figure 34. Plasma cortisol levels of juvenile fall chinook salmon sampled from McNary Dam after 2 d recovery in the raceway after collection (R2) and after loading into transport truck (PL), and during 8 d of post-transport recovery at Bonneville Dam. Samples were taken June 16-24 (early run), July 14-22 (mid run), and August 2-10 (late run), 1982. On August 20, 1982, fish were sampled from a transport truck and after release (⊗). All points represent mean + SE for n = 11 to 14. Points marked (a) are significantly different from Time = 0 of the same line (LSD test, P < .05).
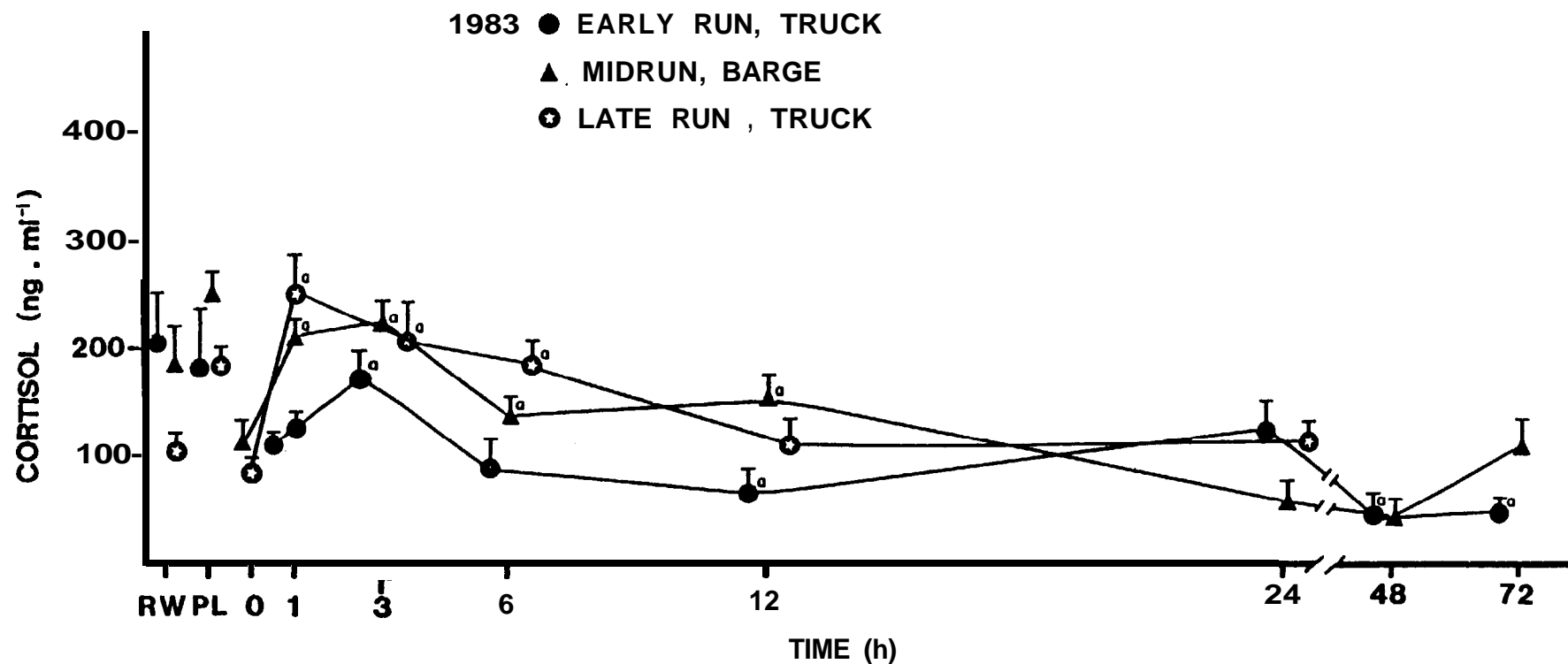
Figure 35. Plasma cortisol of juvenile fall chinook salmon sampled from a raceway at McNary Dam just prior to being loaded into a truck or barge (RW), after being loaded onto a truck or barge (PL), and after a 3- to 4-h truck transport or 15- to 16-h barge transport to Bonneville Dam. Fish were also removed from the transport vehicle, held in plastic buckets (ca. 100L) with flow-through water, and serially sampled through 72 h. All points are means + SE of 10 to 12 fish. Points marked (a) are significantly different from Time = 0 of the same line (P < .05, LSD test).
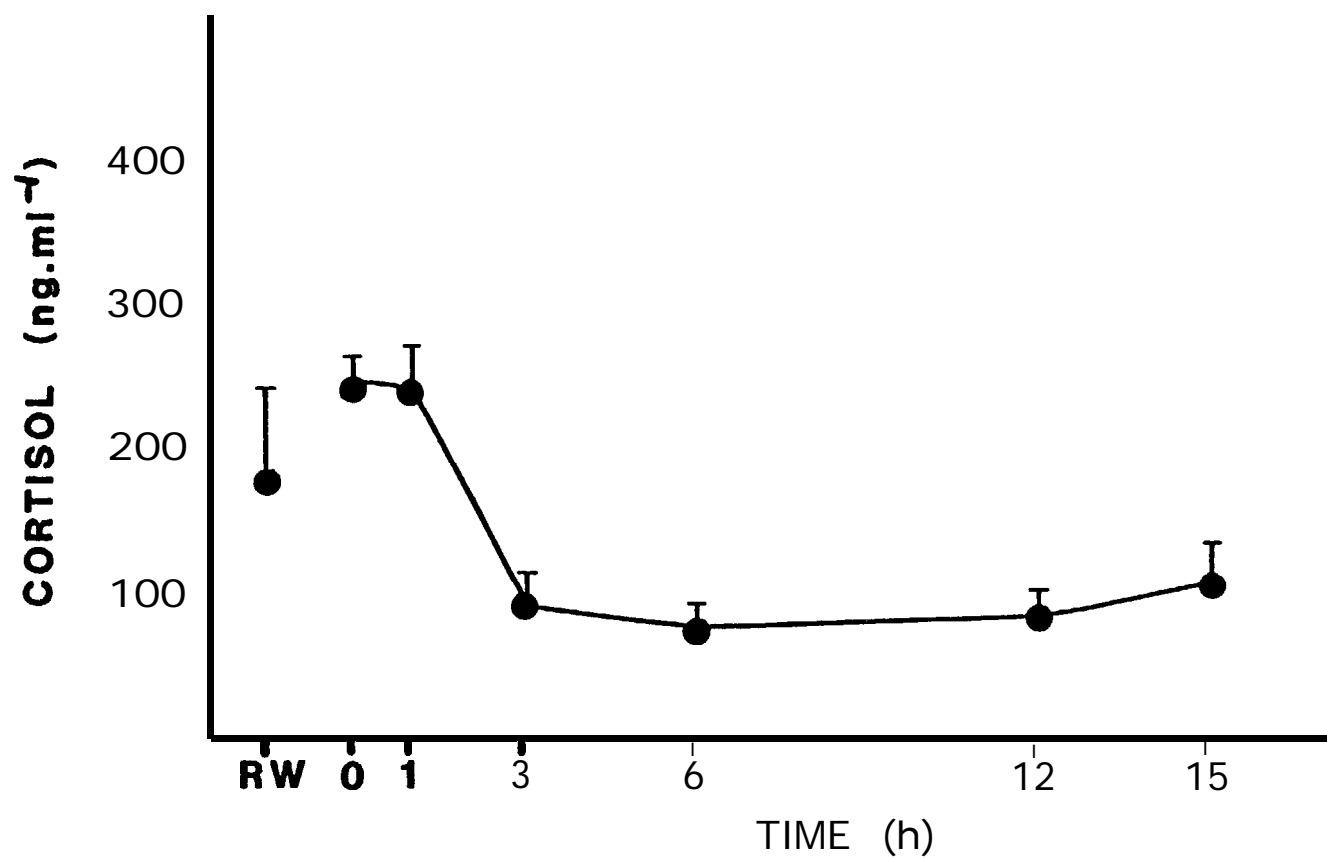
Figure 36.   Plasma cortisol of juvenile fall chinook salmon collected from a McNary Dam raceway (RW) just before being loaded into a transport barge and throughout transport to Bonneville Dam on July 9, 1983.   All points are the mean + SE of 10 to 12 fish.

this difference reflected conditions in the holding facilities and is not indicative of differences in transportation. It appears that the plasma cortisol dynamics of fish removed from a transport truck at Bonneville Dam are best described by the 1983 results (Fig. 35), in that cortisol levels increase to a peak within 3 to 6 h and then decrease to baseline levels within 24 h.

Interrenal cell nuclear diameters of transported fish did not change significantly during 4 to 8 d after transport (Fig. 37). Liver glycogen of fish transported by truck (Fig. 38) was generally lower than that of fish sampled at McNary Dam (Fig. 13) and declined to almost negligible levels within 8 d. Hematocrit values of transported fall chinook (Fig. 39) were more variable than those of fish at McNary Dam (Fig. 18). The 1983 results were unusual in that there was almost a 10% difference in hematocrits of fish prior to transport comparing early run to mid-run (Fig. 40). However, in all of the hematocrit data (Figs. 13, 14, 39, and 40) after some recovery, the values are approximately 50%. The relative numbers of WBC in fish transported to Bonneville Dam do not exhibit any strong trends, but it appears that WBC number increases through time after transport (Figs. 40 and 41).

Saltwater challenges of fall chinook transported in 1982 indicated that osmoregulatory ability was reduced in fish as the run progressed and the longer period of time that fish were held after transport (Fig. 42). There is some indication that mid-run fall chinook were better able to osmoregulate than fish from other portions of the run as this group was the only group which grew during the 15 d saltwater growth experiment at the
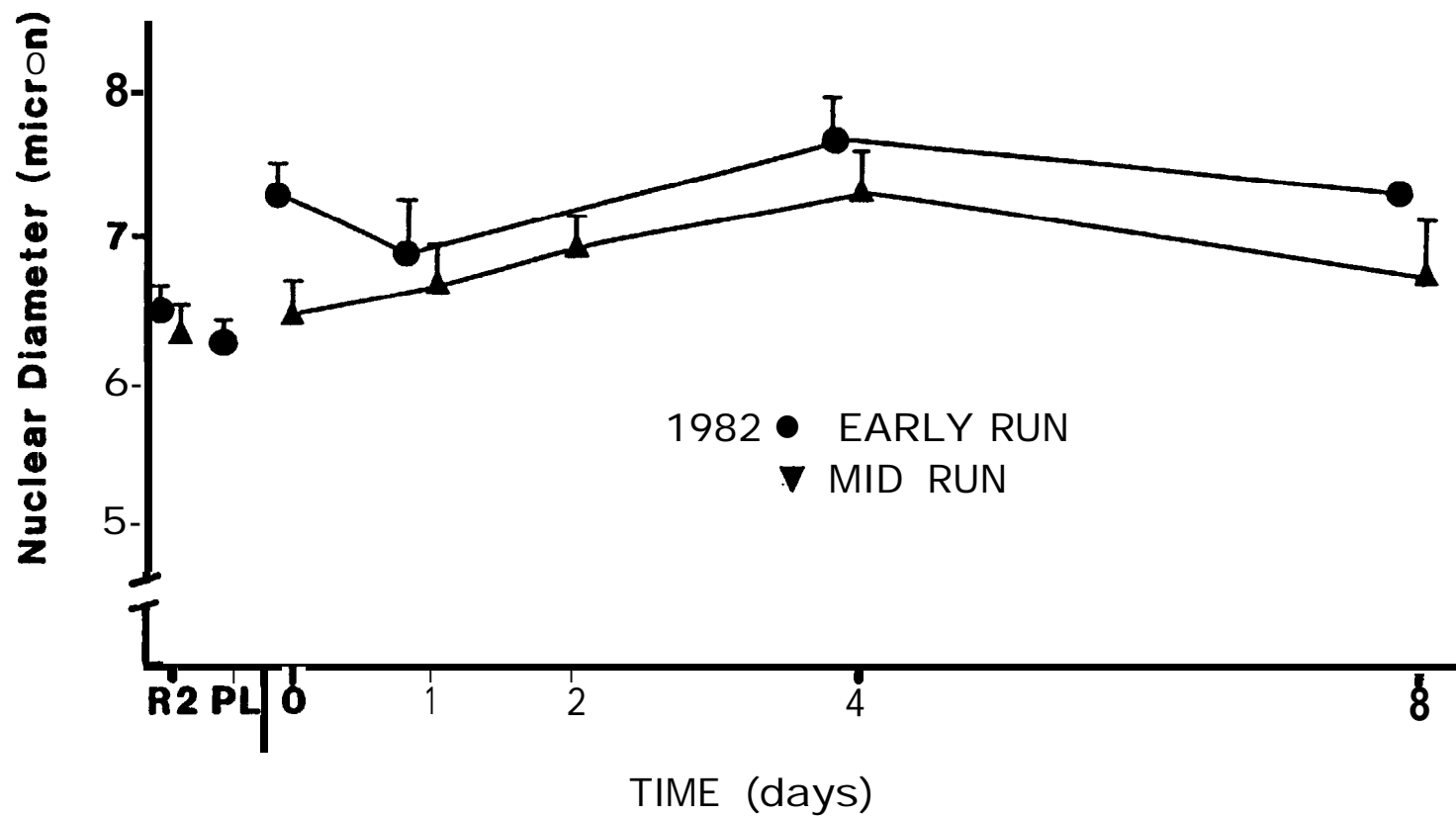
Figure 37. Interrenal cell nuclear diameters of juvenile fall chinook salmon collected after they had been in a raceway at McNary Dam for 2 d (R2), after the fish had been loaded into a transport truck (PL), and for up to 8 d after transport to Bonneville Dam. Samples were collected June 14-20 (early) and July 7-16 (mid), 1982. All points are the means + SE for 4 to 6 fish.
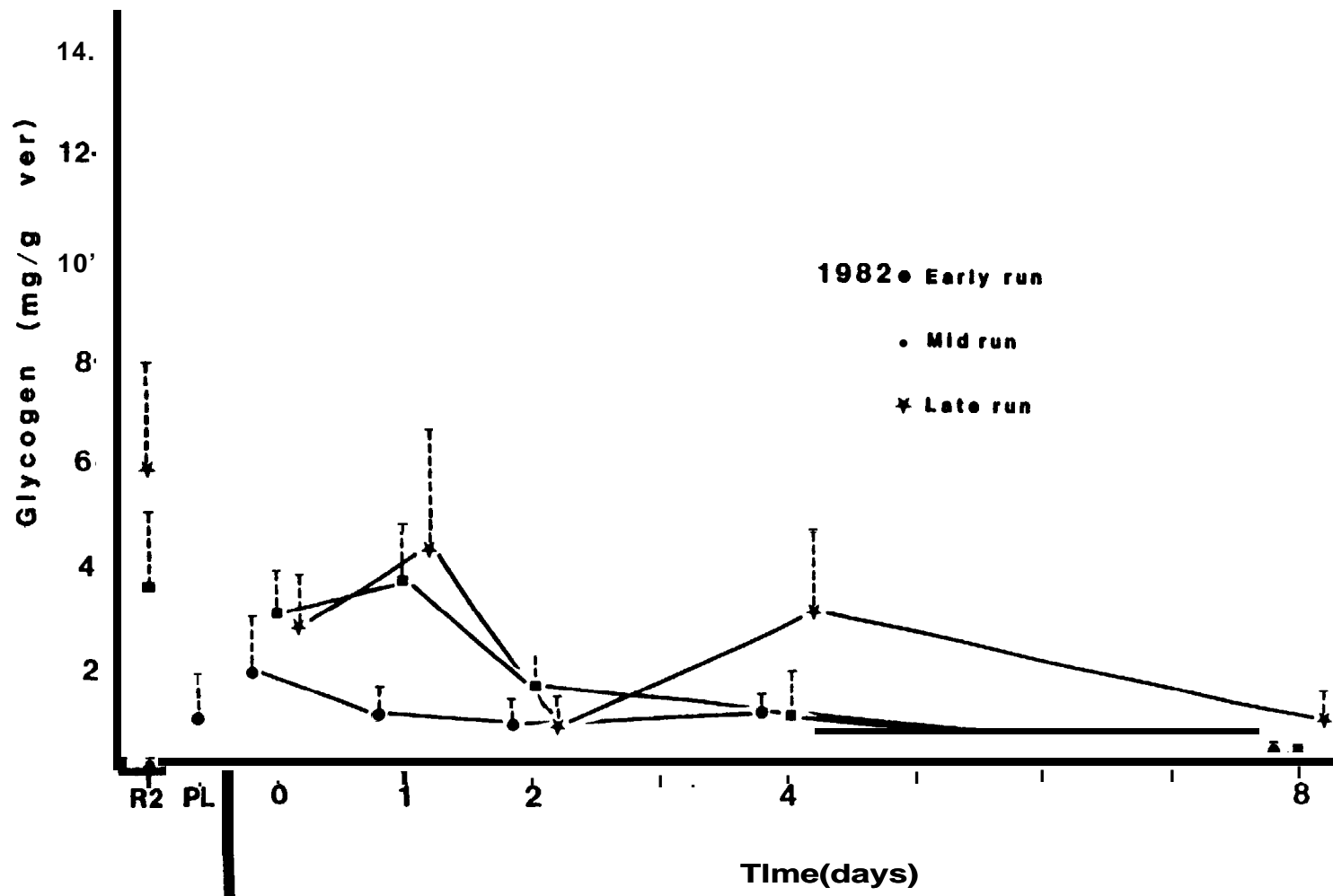
Figure 38. Liver glycogen levels of juvenile fall chinook salmon sampled from the McNary Dam after 2 d of recovery in the raceway (R2), after loading into transport truck (PL), and during 8 d of post-transport recovery at Bonneville Dam. Samples were taken June 16-24 (early run), July 14-22 (mid run), and August 2-10 (late run), 1982. All points represent mean + SE for n = 5 or 6.
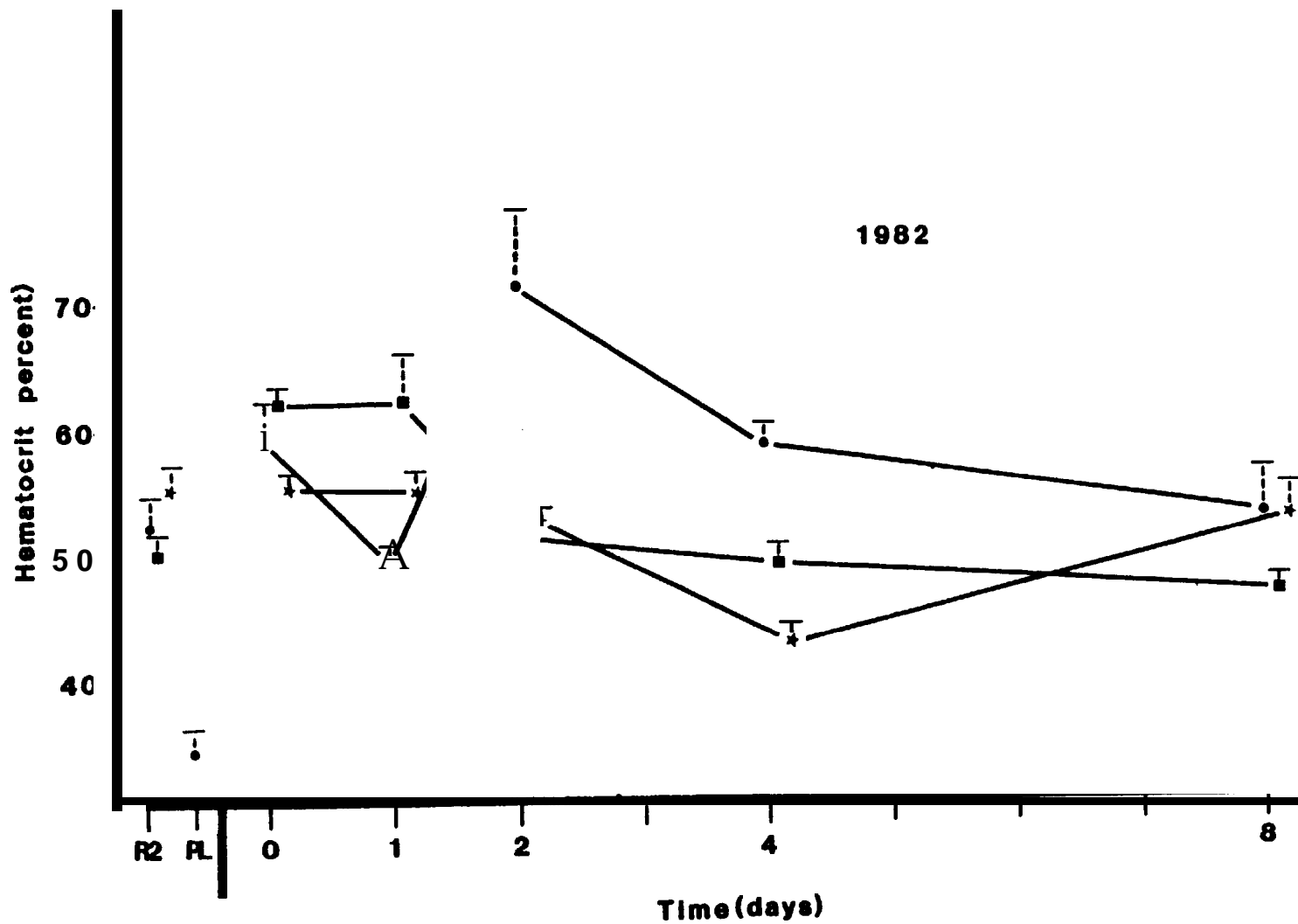
Figure 39. Bematocrit values (mean + SE) for outmigrant fall chinook salmon sampled after 2 d recovery in a raceway (R2) after being loaded into a truck (PL) at McNary Dam, and through 8 d of recovery after being transported to Bonneville Dam. Each point represents 6 fish taken on June 16-24 (●), July 14-22 (■), and August 2-10 (✱), 1982.
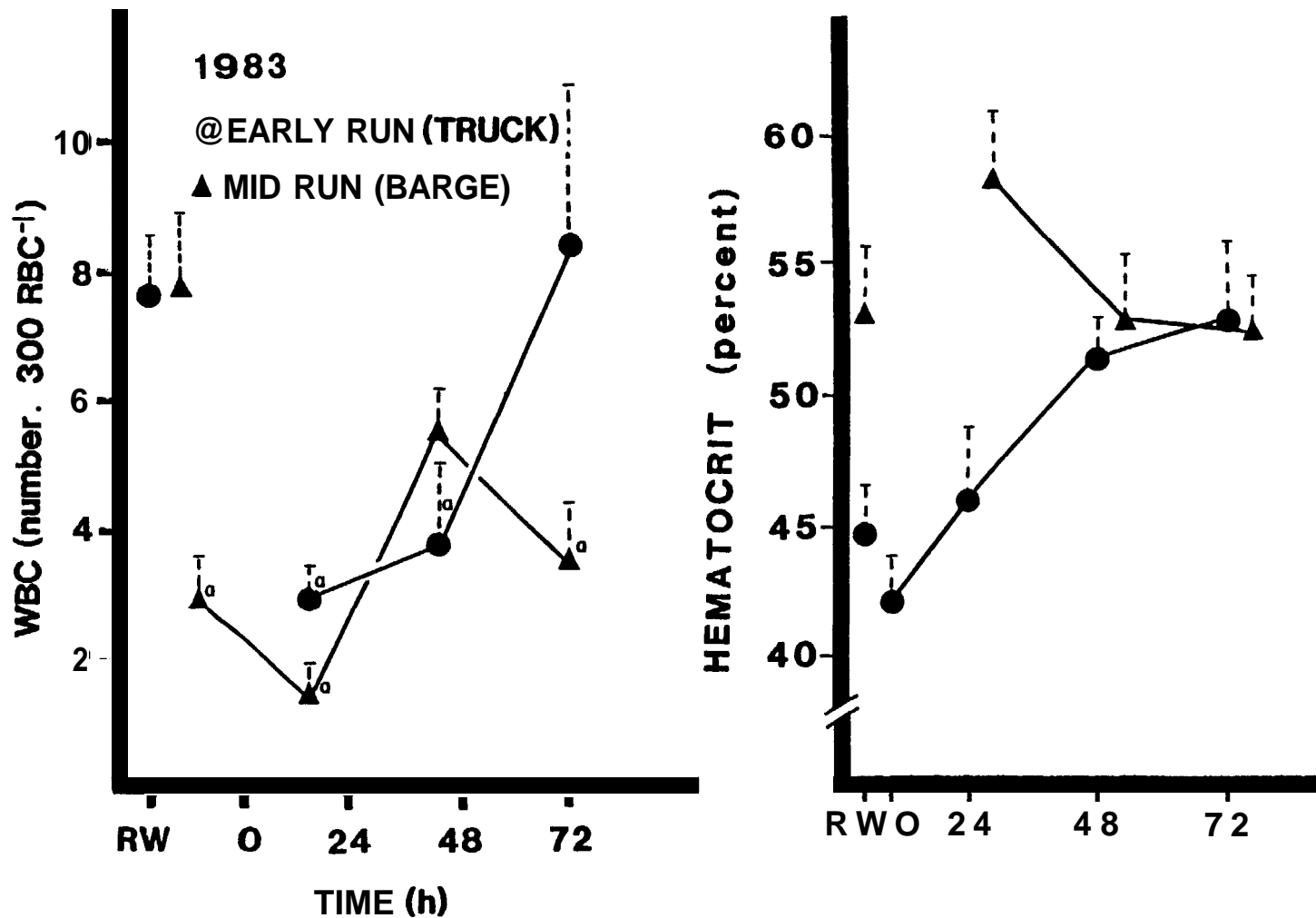
Figure 40. White blood cell (WBC) counts and hematocrit values in juvenile fall chinook salmon collected from a raceway at McNary Dam just prior to being loaded into a transport vehicle (RW) and for up to 72 h after being transported to Bonneville Dam. Sampling was done during June 14-24 (early run) and July 7-16 (mid run), 1983. All points are the means + SE for 5 to 6 fish. WBC counts are the average of two replicate counts of the number of WBC among 300 erythrocytes (RBC) on blood smears. Points marked (a) are significantly different from raceway values with the same symbol (P < .05, LSD test).
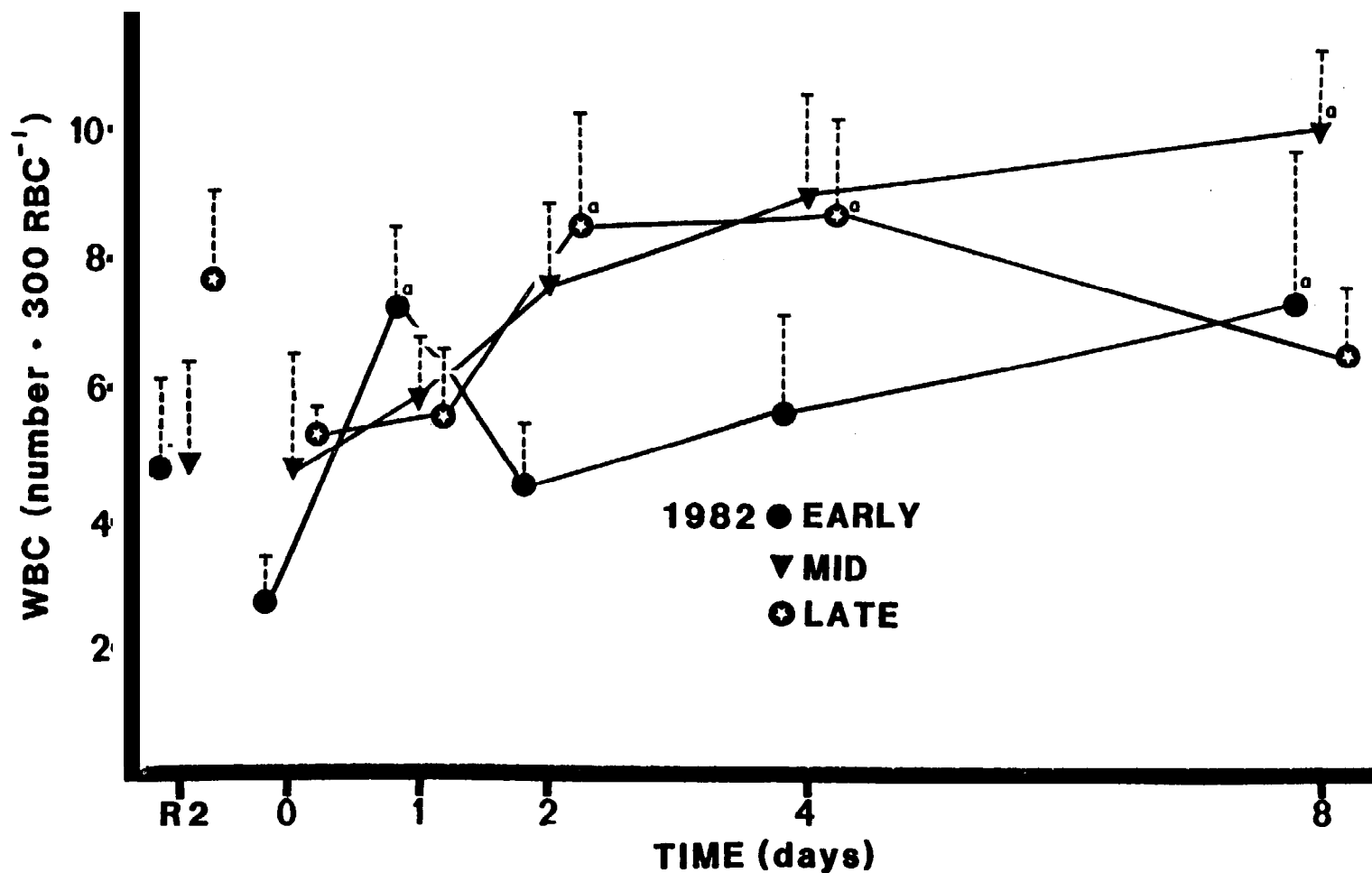
Figure 41. White blood cell (WBC) counts of juvenile fall chinook salmon collected after being in a raceway at McNary Dam for 2 d (R2), after transport, and for up to 8 d after transport to Bonneville Dam. Sampling was during June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982. All points are means + SE of the average of two replicate counts of the number of WBC's among 300 erythrocytes (BBC) on blood smears of 6 fish. Points marked (a) are significantly different from those of Time = 0 of same line (P < .05, LSD test).
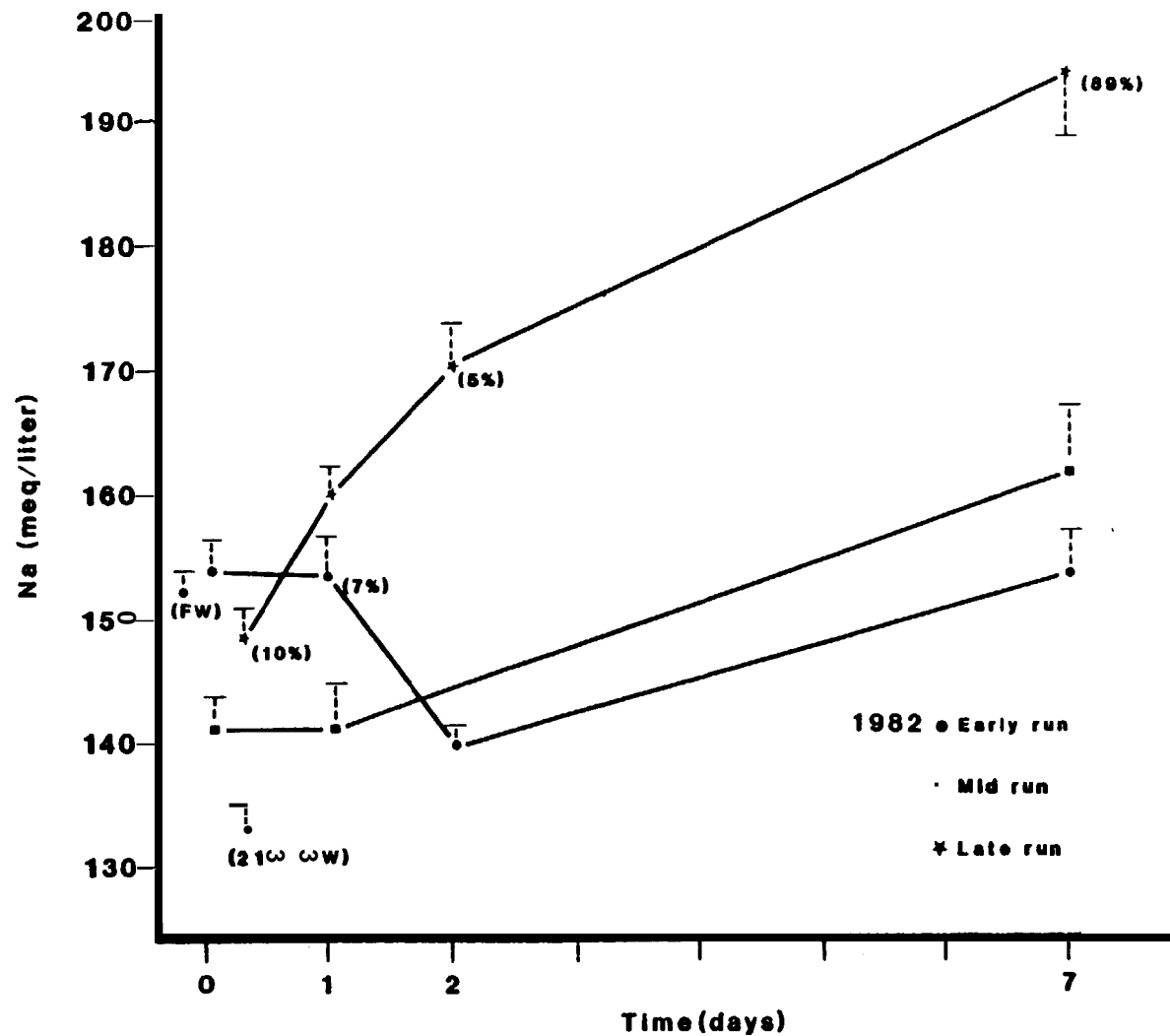
Figure 42   Plasma Na levels (mean + SE) of outmigrant fall chinook salmon sampled after transport from McNary Dam and during 8 d of recovery at Bonneville Dam. All points are duplicates of 10 fish which were sampled 24 h after being put in 15 parts per thousand salt water or fresh water (FW) during June 16-24 (early run), July 14-22 (mid run), and August 2-10 (late run), 1982. Percent mortality is in parentheses.

Marine Science Center. In 1983, there was no difference in osmoregulatory ability of early-run fish transported by truck compared to mid-run fish transported by barge, which confirmed our conclusion based on plasma cortisol that there was no difference between truck and barge transport (see: Barge transport). The secondary stress challenge of truck-transported fall chinook resulted in a three-fold greater maximum plasma cortisol response in 1982 than in 1983 (Figs. 20 and 43); however, in both years, within 6 h plasma cortisol levels declined to levels equivalent to those of fish prior to transport and prior to the secondary stress.

Disease challenge of transported fish. In general, fish allowed 1 d of recovery after transport from McNary Dam resisted Vibrio anguillarum longer than did fish exposed immediately after transport, or after 8 d of recovery (Table 1). Those results parallel the 1982 saltwater challenge data, which indicated a decreased ability to osmoregulate after fish had been held 7 or 8 d (Figs. 18 and 42). The stress of transport and handling could explain the decreased ability to withstand V. anguillarum initially. Prolonged holding of migrating fish may also have caused increased stress, which decreased the fishes' ability to resist V. anguillarum.

Seawater growth and survival. The lengths and weights of transported fish introduced into sea water changed very little in any of three trials (Table 2). The only statistically significant changes were reductions in weight during the early and late portions of the run; however, fish in the mid-run test did show some growth, and the acclimated hatchery fish showed significant growth in length and weight during the 30 d test period
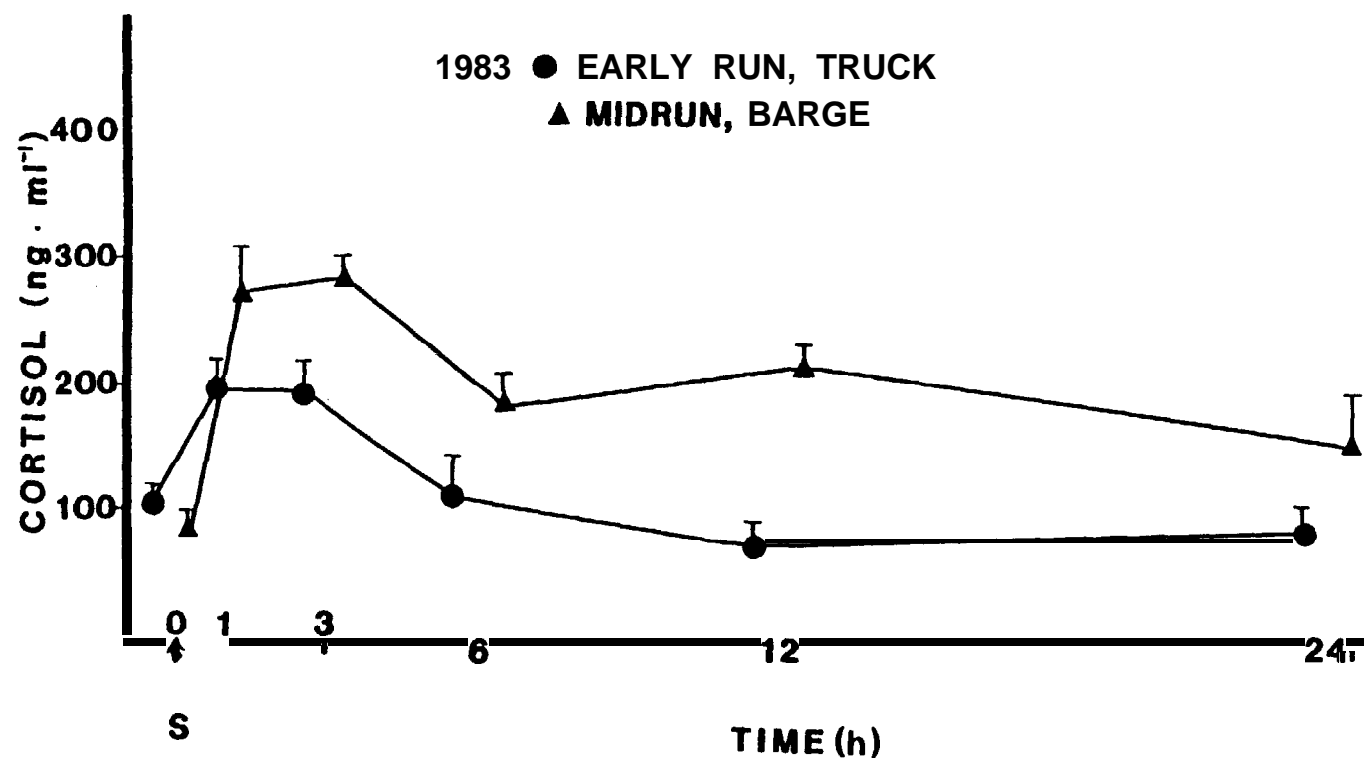
Figure 43. Plasma cortisol levels in juvenile fall chinook salmon which were transported by truck or barge from McNary Dam to Bonneville Dam, and then secondarily stressed by being held in a dipnet out of water for 30 s. Fish were then held in a plastic tank (ca. 100L) and serially sampled through 24 h. All points are means + SE for 10 to 12 fish.

Table 1. Mean of the time to death (MTD) = $\dfrac{(\text{mortalities*day-l}) (\text{total days} = 113)}{\text{total mortalities}}$ and percent total mortalities

for juvenile fall chinook exposed to <u>Vibrio anguillarum</u> in fresh water (FW) or salt water (SW)

after transport from the McNary Dam fingerling collection facility, 1982. A and B are replicate

trials. Numbers of fish at the time of the <u>Vibrio</u> challenge are in parentheses.

Dates	Post-transport Days to Challenge	FW (A)		FW (B)		SW (A)		SW (B)	
		MTD	% Mortality	MTD.	% Mortality	MTD	% Mortality	MTD	% Mortality
Early Run									
June 17–	0	3.76	65(26)	4.12	62(26)	4.10	91(23)	3.52	100(27)
July 8	1	8.62	62(26)	6.67	50(18)	6.10	79(24)	6.67	84(25)
	8	5.73	69(32)	3.76	71(24)	6.67	90(10)a	6.05	86(22)a
Mid-Run									
July 9 –	0	4.79	96 (25)	8.11	93 (30)	4.10	75(24)	3.81	64(25)
July 30	1	6.96	100(25)a	5.70	100(24)	4.07	65(23)	4.92	54(24)
	8	2.30b	100(10)a	1.76b	100(17)a	3.17	57(21)	4.80	83(17)a
Late Run									
Aug. 9 –	0	3.59	76(29)	5.17	93(28)	3.87	96(25)	4.70	89(26)
Aug. 31	1	4.36	76(29)	7.56	86(29)	6.43	78(27)	5.82	a
	8	3.26	74(27)	2.56	93 (29)	2.70	95(17)a		74(27)

[a]pre-challenge mortalities > 18%

[b]100% mortality before day 13 of challenge

Table 2. Fork length (FL), weight, condition factor ($K = \frac{g \times 100}{cm^3}$), percent weight change and mortality of fall chinook transported from McNary Dam fingerling facility and introduced into seawater at the Marine Science Center for 15 or 30 days. A and B are replicate trials.

Dates	Treatment		Final N	FL (cm) (mean+SE)	Weight(g) (mean+SE)	K	Werght change(%)	Mortality %	MTD[b]
Early run									
June 18	Transported	Initial	76	9.4+0.2	9.7+0.4	1.0			
Jul 2	15 day	A	52	9.3+0.2	8.2+0.4a	1.0	(-15.5)	39	1.0
		B	40	9.5+0.2	8.7+0.6	1.0	(-10.3)	39	1.1
Mid-run									
Jul 8	Transported	Initial	30	9.4+0.2	8.2+0.7	1.0			
Jul 23	15 day	A	44	9.7+0.2	9.5+0.4	1.0	15.9	0	
		B	45	9.6+0.2	9.1+0.4	1.0	11.0	0	
Late run									
Aug 9	Transported	Initial	30	11.7+0.1	18.7+0.6	1.2			
Aug 24	15 day	A	41	11.4+0.3	16.7+0.5a	1.1	(-10.7)	10	2.5
		B	45	11.6+0.1	16.6+0.5a	1.1	(-10.2)	11	2.5
Sept 9	30 day	A	13	11.9+0.2	16.5+1.0	1.0	(-9.6)	68	
		B	26	11.6+0.2	15.7+0.7A	1.0	(-16.0)	42	
Aug 9	Acclimated[c]	Initial	30	9.4+0.1	9.6+0.4	1.2			
Aug 24	15 day	A	14	9.7+0.3	10.0+0.7	1.1	4.2	30	9.4
		B	21	9.6k0.2	10.2+0.7	1.2	6.3	33	8.4
Sept 9	30 day	A	6	10.8+0.4A	14.8+2.0A	1.2	54.2	71	
		B	11	10.3+0.4A	12.7+1.7A	1.2	32.3	48	

[a] Value significantly different from initial value (P>.0l, t-test).

[b] Mean of the time to death = $\dfrac{(mortalities.day\text{-}l)\ (total\ days = 15)}{total\ mortalities}$

'Juvenile fall chinook obtained from Trask Fish Hatchery, Tillamook, Oregon on July 23, 1982 and held at MSC in freshwater and switched to seawater on August 9.

(Table 2). During tests, the transported fish ate little or no food in comparison with the hatchery fish. This failure to eat can account for the loss in weight of the transported fish.

Mortality was very high in fish acclimated to the experimental setting (ca. 30% after 15 d, and 48 to 71% after 30 d) and in fish transported from McNary Dam (0 to 39% after 15 d, and 42 to 68% after 30 d). However, the mean of the time to death (MTD) was earlier in transported fish (1.0 to 2.6 d) when compared to acclimated fish (MTD = 8.4-9.4), suggesting osmoregulatory failure. There were no mortalities among the fish transported in July which is the time of peak abundance of emigrating fall chinook on the Columbia River (Basham et al. 1983). Na-K ATPase was significantly higher during this time (Fig. 8), and fish transported to Bonneville Dam and challenged with salt water immediately upon arrival were better able to regulate plasma Na levels during the mid-run than the early- or late-run fish (Fig. 42). All of this suggests that fall chinook smolts emigrating in the mid-run were at an osmoregulatory optimum for entry into sea water after transportation.

Transported fish density. Loading density did not have a discernable effect on plasma cortisol levels in fall chinook immediately after they were loaded into Corps of Engineers' trucks (Fig. 44a,b). Again, plasma cortisol levels were lower after 3 to 4 h of transport than immediately after fish were loaded into the truck; however, it appears that the absolute reduction was greatest at low densities. For example, late-run fish were transported at 0.05 $lbs \cdot gal^{-1}$, and plasma cortisol decreased from approximately 390 $ng \cdot ml^{-1}$ to 190 $ng \cdot ml^{-1}$, a reduction of 200 $ng \cdot ml^{-1}$ (Fig. 44a). During the mid-run, plasma cortisol in fish transported at 0.5 and .08 lbs.gal-1 decreased by
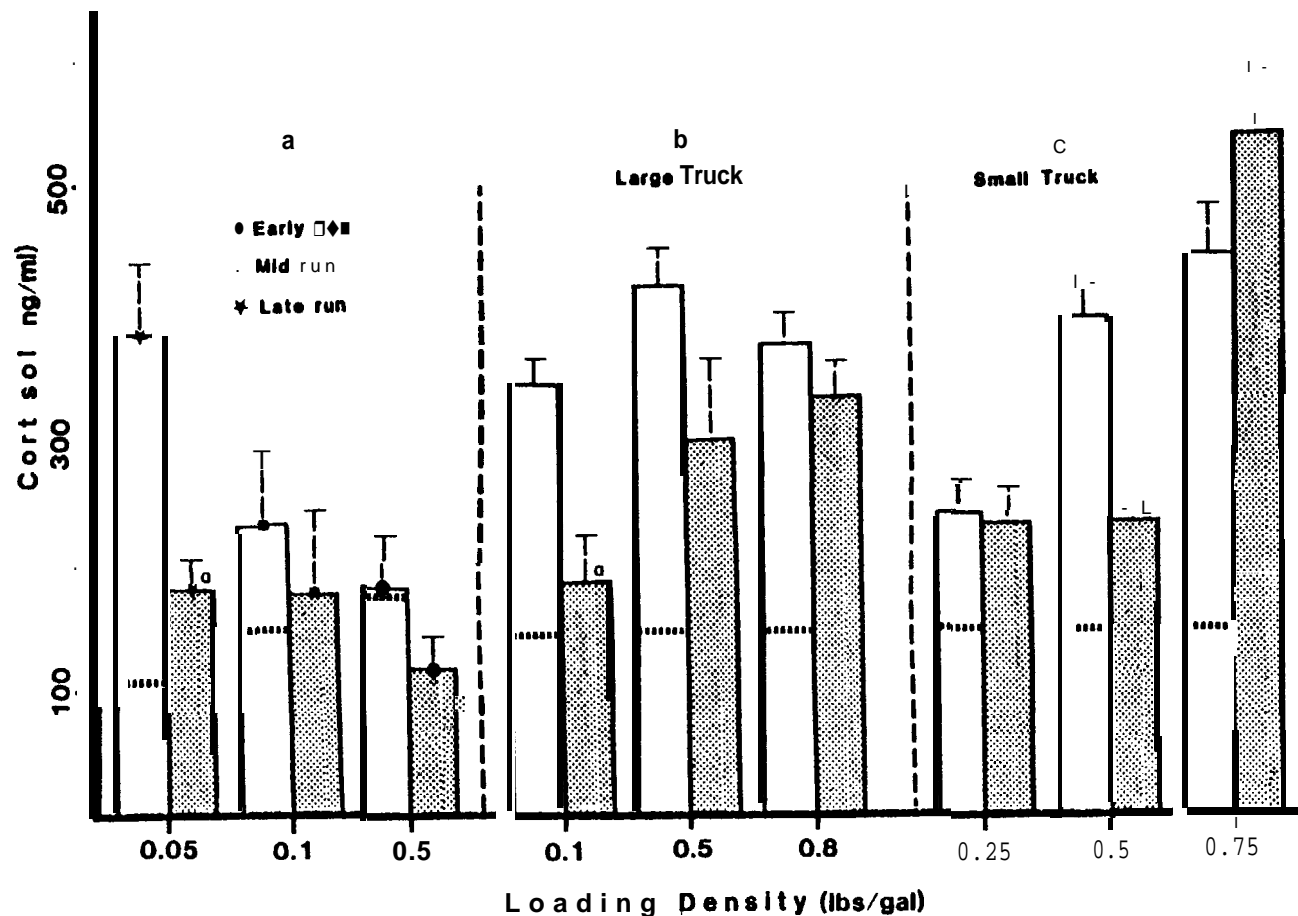
Figure 44. Plasma cortisol levels (mean + SE) of juvenile fall chinook salmon immediately after being loaded into a transport truck at McNary Dam (clear bars) and after 3-4 h of transport to Bonneville Dam (stippled bars). Sections of the figure represent (a) fish sampled from normal transportation operations throughout the season, (b) late-run fish loaded at various densities in a single truck partitioned into three cells, and (c) late-run fish loaded into a small, 200-gallon pickup truck mounted tank. Dashed line in clear bars are cortisol levels of fall chinook after 2 day of recovery in the raceway after collection at McNary Dam. All sample sizes are 11 to 14 fish. Values marked (a) are significantly different from values of fish immediately after being loaded (p < .05, LSD test).

125 and 40 $ng \cdot ml^{-1}$ (Fig. 44b), respectively, even though the cortisol levels immediately after loading were approximately the same, 375 to 425 $ng \cdot ml^{-1}$. These results also indicate that the use of the small transport truck does not satisfactorily simulate the environment of the Corps of Engineers' truck. Although plasma cortisol levels are similar in fish immediately after they were loaded on the small truck, the reduction in cortisol levels is not comparable to that in fish transported in the Corps of Engineers' truck. In fact, at the highest density in the small truck, there was an increase in plasma cortisol in fish during transport (Fig. 44c).

Barge transport. The plasma cortisol dynamics of fish removed from the barge at Bonneville Dam during the 1983 mid-run were very similar to those of fish trucked to Bonneville Dam during the early and late portions of the run (Fig. 35). In fact, plasma cortisol levels in fish sampled from the barge while enroute were reduced to pre-stress levels within 3 h after the fish were loaded into the barge (Fig. 36), indicating that recovery from the stress of being loaded into the barge was similar to the recovery from being loaded into the truck. There were no differences in hematocrit or WBC numbers that could be attributed to the different transport vehicles (Fig. 40), nor was there a difference in osmoregulatory ability between trucked and barged fish (Fig. 19B). However, fish transported by barge had higher plasma cortisol levels in response to the secondary stress challenge than did fish transported by truck, and the cortisol did not return to pre-stress levels within 24 h in fish transported by barge (Fig. 43). This may reflect some unusual factor in the particular barge holding-tank from

which we sampled fish, as unusually high mortalities (ca. 30%) were noted when the barge crew released those fish downriver (Brad Eby, U.S. Army Corps of Engineers, personal communication).

STRESS CHARACTERIZATION STUDIES

Multiple Stresses

Experimental design, results, and discussion. We conducted laboratory
experiments to determine physiological responses to standardized multiple
stresses. These permitted a better understanding of physiological responses
to repeated stresses which a fish might encounter in various elements of the
collection system, and assisted in interpretation of the clinical indices of
stress data obtained from fish at McNary Dam and after transport. Juvenile
fall chinook salmon obtained from Trask Hatchery, Tillamook, Oregon, were
held under constant conditions (i.e., fed ca. 1.5% initial body $wt \cdot d^{-1}$;
water temperature ca. 12.5 C) at the Oregon State University Smith Farm
facility in Corvallis. In the experiments, tank acclimated fish (FL 11.5 cm,
wt 16.6 g; 2 wk in tanks at 4 L min^{-1} inflow) were exposed to the
standardized handling stress consisting of dip-netting the fish from the
tank and suspending them in the air for 30 s before returning them to an
identical tank. Experiments were conducted with 6 fish each from duplicate
tanks and the results were pooled (n = 12).

The physiological responses to a single standard stress were determined
at 0, 0.5, 1, 3, 6, 12, and 24 h after the 30-s stress. Then, to determine
effects of subsequent stresses, fish were stressed twice with a 3-h interval
between, and another group of fish was stressed three times at 3-h
intervals. In another series of experiments, fish were subjected to
two 30-s handling stresses spaced 1, 3, or 12 h apart, and then serially
sampled over 24 h. At each monitoring time, plasma and tissue samples
were collected for later analysis of plasma cortisol, glucose and lactate,
and hepatic glycogen.

Plasma cortisol in juvenile chinook reached a maximum of 182 $ng \cdot ml^{-1}$ in 0.5-3 h, and returned to control levels within 6 h when the fish were exposed to a single acute, but severe, 30-s handling stress (Fig. 45). In fish subjected to a second and third identical handling stress 3 h and 6 h after the initial handling stress, cortisol response followed the same pattern, but peak levels of 296 and 476 $ng \cdot ml^{-1}$, respectively, were additive upon prior responses. In all cases, cortisol declined to control levels within 24 h (Fig. 45). Similarly, levels of plasma glucose were additive upon prior responses. Peak levels of glucose occurred 3-6 h after application of the final stress, and were 83.8, 133.5 and 204.2 $mg \cdot 100\ ml^{-1}$ for the single stress, two handling stresses and three stresses spaced 3 h apart, respectively (Fig. 46). Plasma lactate levels rose rapidly to 51.0 $mg \cdot 100\ ml^{-1}$ within 0.5 h after a single stress; lactate levels exhibited a similar response to a second stress at 3 h, but remained high for a longer period of time (Fig. 47). Plasma lactate levels after the initial stress, and the second stress in the triple-stress experiment were not consistent with the previous responses. The response to the third stress at 6 h was similar in pattern to that from a single stress, with the absolute peak response of 76.3 $mg \cdot 100\ ml^{-1}$ occurring at 0.5 h (Fig. 47). In fish subjected to the second stress at 3 h and fish subjected to the second and third acute stresses at 3 h and 6 h, minimum liver glycogen levels occurred at 6 h after the initial stress (Fig. 48). In both groups, liver glycogen decreased from greater than 30 $mg \cdot g^{-1}$ to less than 6 $mg \cdot g^{-1}$. However, fish that were subjected to the single initial handling stress did not demonstrate any consistent changes in liver glycogen (data not shown).
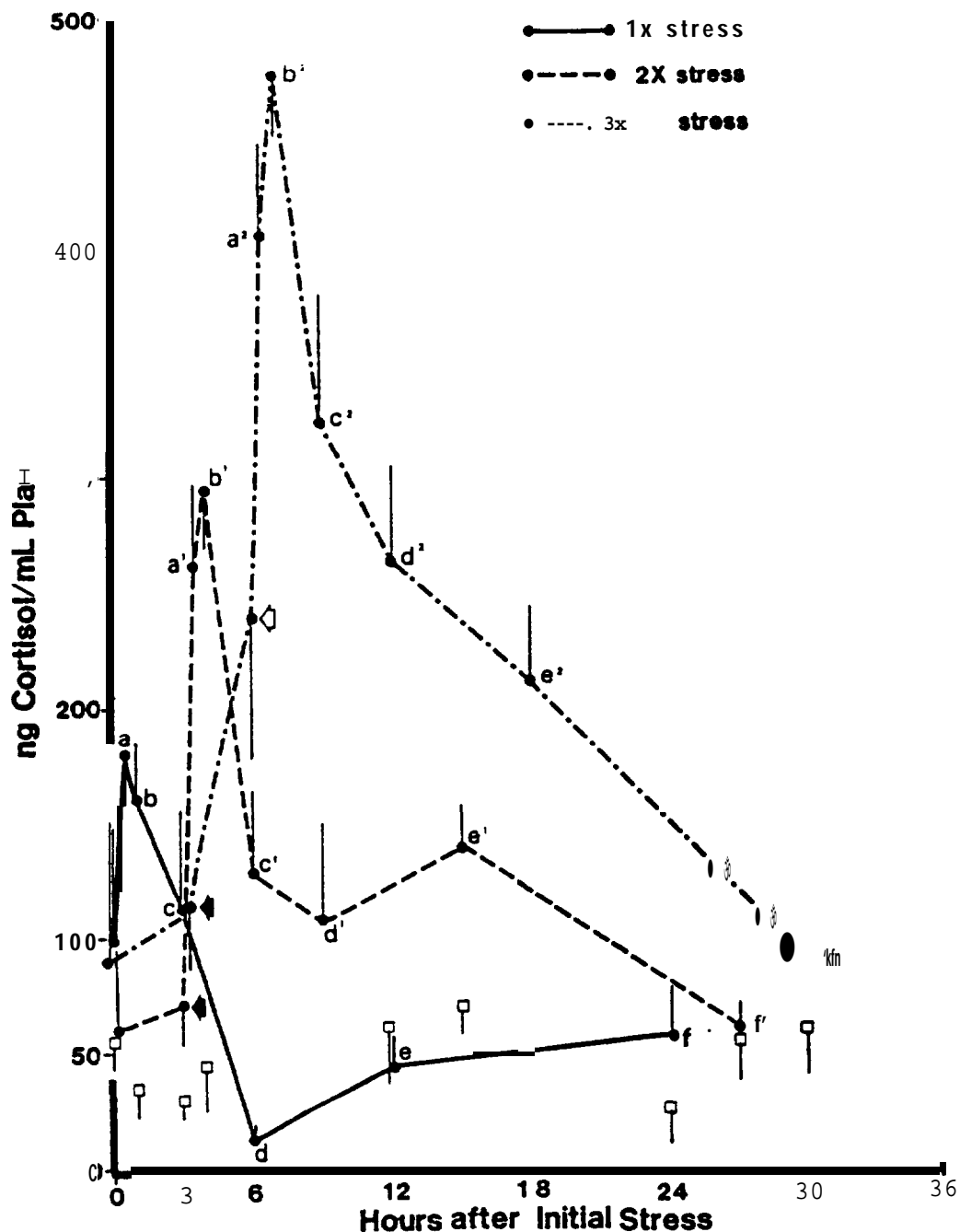
Figure 45. Plasma cortisol (ng/mL+/-SE) in juvenile fall chinook salmon
subjected to a single (1X) 30-s handling stress, or to two (2X)
or three (3X) 30-s handling stresses spaced 3 h apart. Sample
sizes of n=12 represent pooled data for duplicate tanks. Open
squares are values for unstressed controls. Mean values for
specific time points after application of the final stress, as
designated by letters on the figure, are ranked as follows from
left to right in order from lowest to highest; means not signi-
ficantly different are underlined (Duncan's new multiple-range
test at 5%):

0.5 h: $\underline{a \quad a^1}$ < a^2 6 h: $\underline{d \quad d^1}$ < d^2

1 h: b < b^1 < b^2 12 h: e < e^1 < e^2

3 h: $\underline{c \quad c^1}$ < c^2 24 h: $\underline{f \quad f^1 \qquad f^2}$

Figure 46. Plasma glucose (mg/100 mL +/-SE) in juvenile fall chinook salmon subjected to a single (1X) 30-s handling stress, or to two (2X) or three (3X) 30-s handling stresses spaced 3 h apart. Sample sizes of n = 12 represent pooled data for duplicate tanks. Open squares are for unstressed controls. Mean values for specific time points after application of the final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

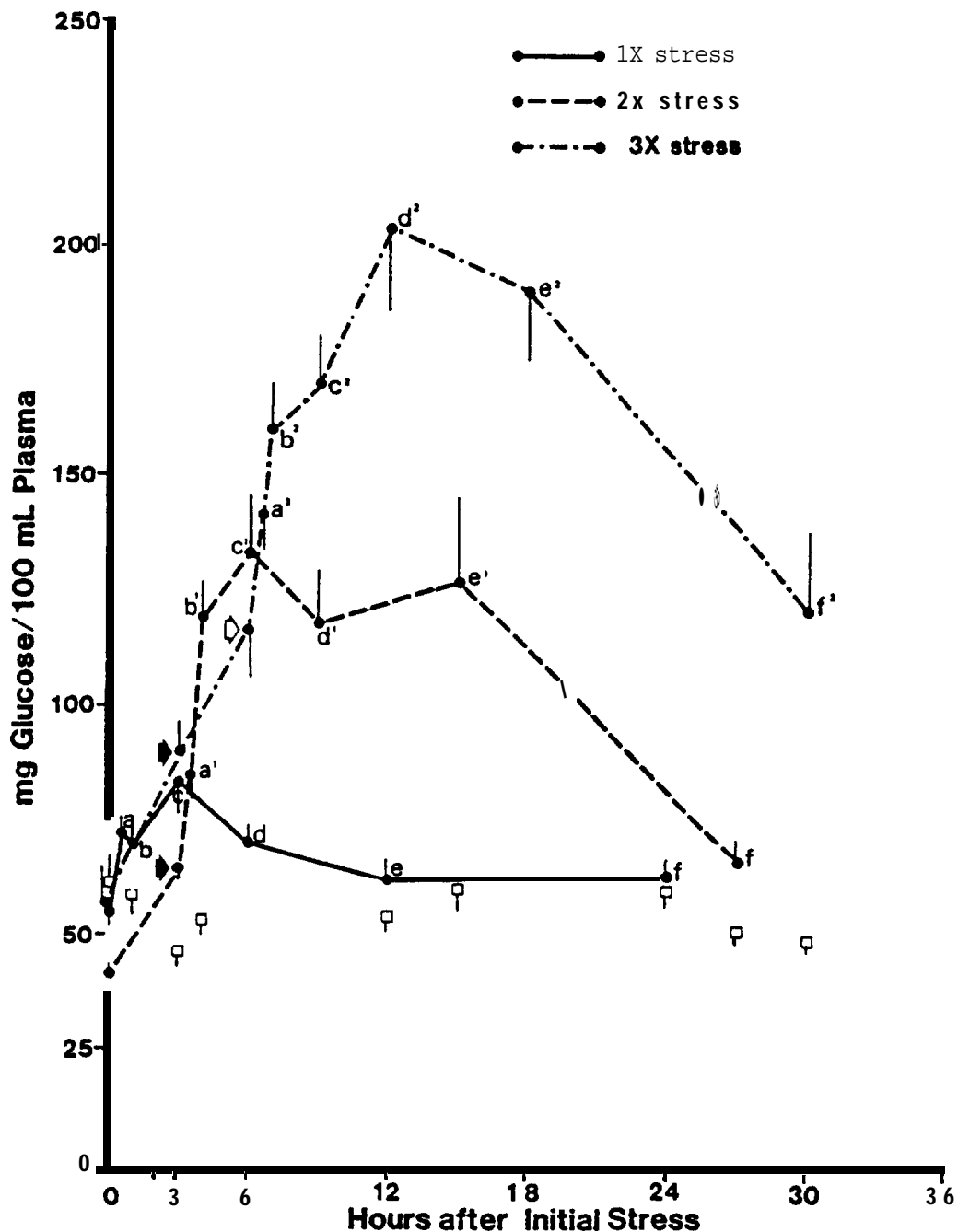|									
0.5 h:	$\underline{a \quad a^1}$	<	a^2		6 h:	d	<	d^1	<	d^2
1 h:	b	<	b^1	<	b^2	12 h:	e	<	e^1	<	e^2
3 h:	c	<	c^1	<	c^2	24h:	$\underline{f \qquad f^1}$	<	f^2

Figure 47.   Plasma lactate (mg/l00 mL +/-SE) in juvenile fall chinook salmon subjected to a single (1X) 30-s handling stress, or to two (2X) or three (3X) 30-s handling stresses spaced 3 h apart.   Sample sizes of n = 9-12 represent pooled data for duplicate tanks.   Open squares are values for unstressed controls.   Mean values for specific time points after application of the final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

0.5 h:	$\underline{a^1}$	$\underline{a}$	$< a^2$
1 h:	$\underline{b^1}$	$\underline{b}$	$< b^2$
3h:	$\underline{c}$	$\underline{c^1}$	c^2
6h:	$d <$	$\underline{d^1}$	$\underline{d^1}$
12h:	$\underline{e}$	$\underline{e^1}$	e^2
24 h:	$\underline{f^1}$	f	f^2
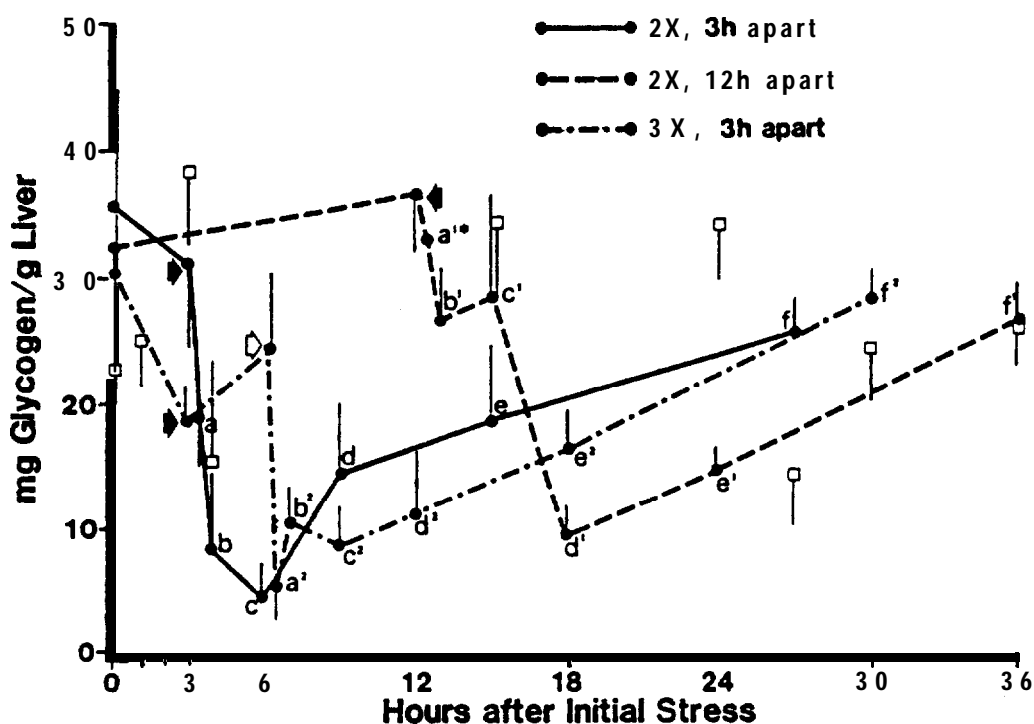
Figure 48. Liver glycogen (**mg/g** +/-SE) in juvenile fall chinook salmon
subjected to two (2X) 30-s handling stresses spaced either
3 h or 12 h apart, or to three (3X) 30-s handling stresses
spaced 3 h apart. Sample sizes of n = 6 represent pooled data
for duplicate tanks. Open squares are values for unstressed
controls. Mean values for specific time points after application
of the final stress, as designated by letters on the figure, are
ranked as follows from left to right in order from lowest to
highest; means not significantly different are underlined
(Duncan's new multiple-range test at 5%):

$$0.5 \text{ h: } \mathbf{a}^2 < a$$

$$1 \text{ h: } \underline{b \qquad b^2} < b^1$$

$$3\text{h: } \underline{c \qquad c^2} < c^1$$

$$6 \text{ h: } \underline{d^1 \qquad d^2 \qquad d}$$

$$12 \text{ h: } \underline{e^1 \qquad e^2 \qquad e}$$

$$24\text{h: } \underline{f \qquad f^1 \qquad f^2}$$

(*Value of $\mathbf{a}^1$ is an estimate using body weights x 1% since
individual liver weights were not available for that sample.)

Fish stressed twice, 1 h apart, had cortisol levels similar to fish stressed twice, 3 h apart; the maximum level of 379 $ng \cdot ml^{-1}$ was cumulative upon the plasma cortisol level of 201 $ng \cdot ml^{-1}$ from the earlier stress (Fig. 49). However, when fish were allowed 12 h to recover, a second stress elicited a much greater relative response in plasma cortisol, i.e., from 64 to 378 $ng \cdot ml^{-1}$ in 0.5 h (Fig. 49). This increased response to a second stress was not evident for plasma glucose, where levels were the same after the second stress independent of the time since the first stress; peak levels after the second stress were 135, 134, and 117 $mg \cdot 100\ ml^{-1}$ for 1 h, 3, and 12 h recovery periods, respectively (Fig. 50). Plasma lactate levels after a second stress were similar to those observed for plasma cortisol. That is, after a 12-h recovery period between stresses, there was a much greater increase in plasma lactate to a maximum level of 98.9 $mg \cdot 100\ ml^{-1}$ in 0.5 h, than there was to a second stress after a 1-h or 3-h recovery period (Fig. 51). When two or three stresses were applied, each separated by 3 h, the minimum liver glycogen levels occurred at 6 h after the initial stress regardless of the application of the second and third stresses. However, the minimum liver glycogen level of 9.5 $mg \cdot g^{-1}$ occurred at 6 h after the second stress when the two stresses were applied 12 h apart (Fig. 48).

These results, particularly for cortisol and glucose, support our hypothesis that certain physiological responses to stress in fish are cumulative. The recovery time between stresses may affect both magnitude and timing of responses to multiple stresses such as changes in cortisol, lactate and glycogen, and that recovery from a single acute stress may take considerably longer than corticoid and glycemic dynamics would indicate. This could be an important factor to consider when making recommendations
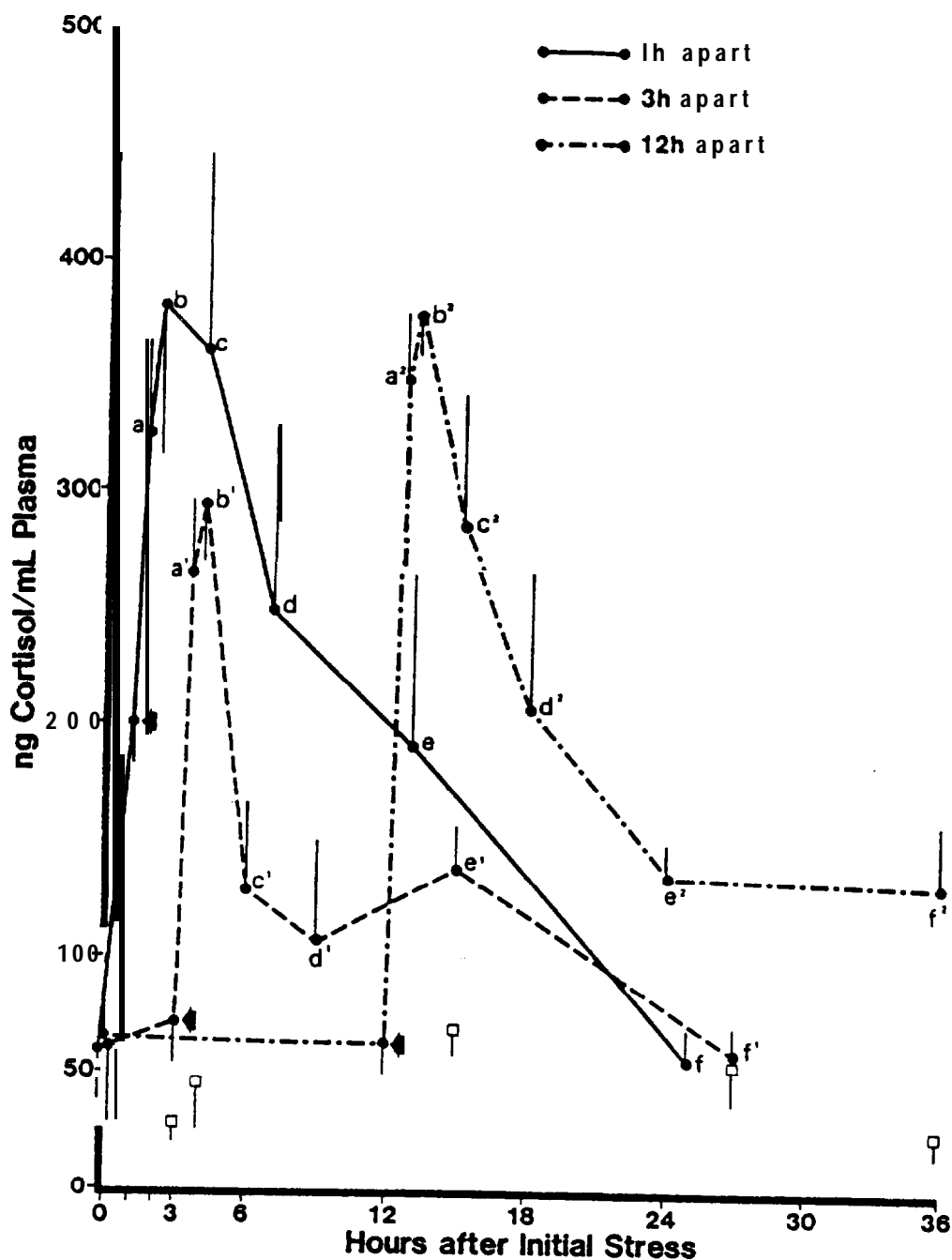
Figure 49. Plasma cortisol (ng/mL +/-SE) in juvenile chinook salmon subjected to two 30-s handling stresses spaced 1, 3, and 12 h apart. Arrows indicate time of second stress. Sample sizes of n=12 represent pooled data for duplicate tanks. Open squares are values for unstressed controls. Mean values for specific time points after application of the final stress, as designated by letters on the figure,are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

0.5 h:	a^1	a	a^2		6 h:	d^1	d^2	d
1 h:	b^2	b^2	b		12 h:	e^2	e^1	e
3 h:	c^1 <	c^2	c		24h:	f	f^1 <	f^2
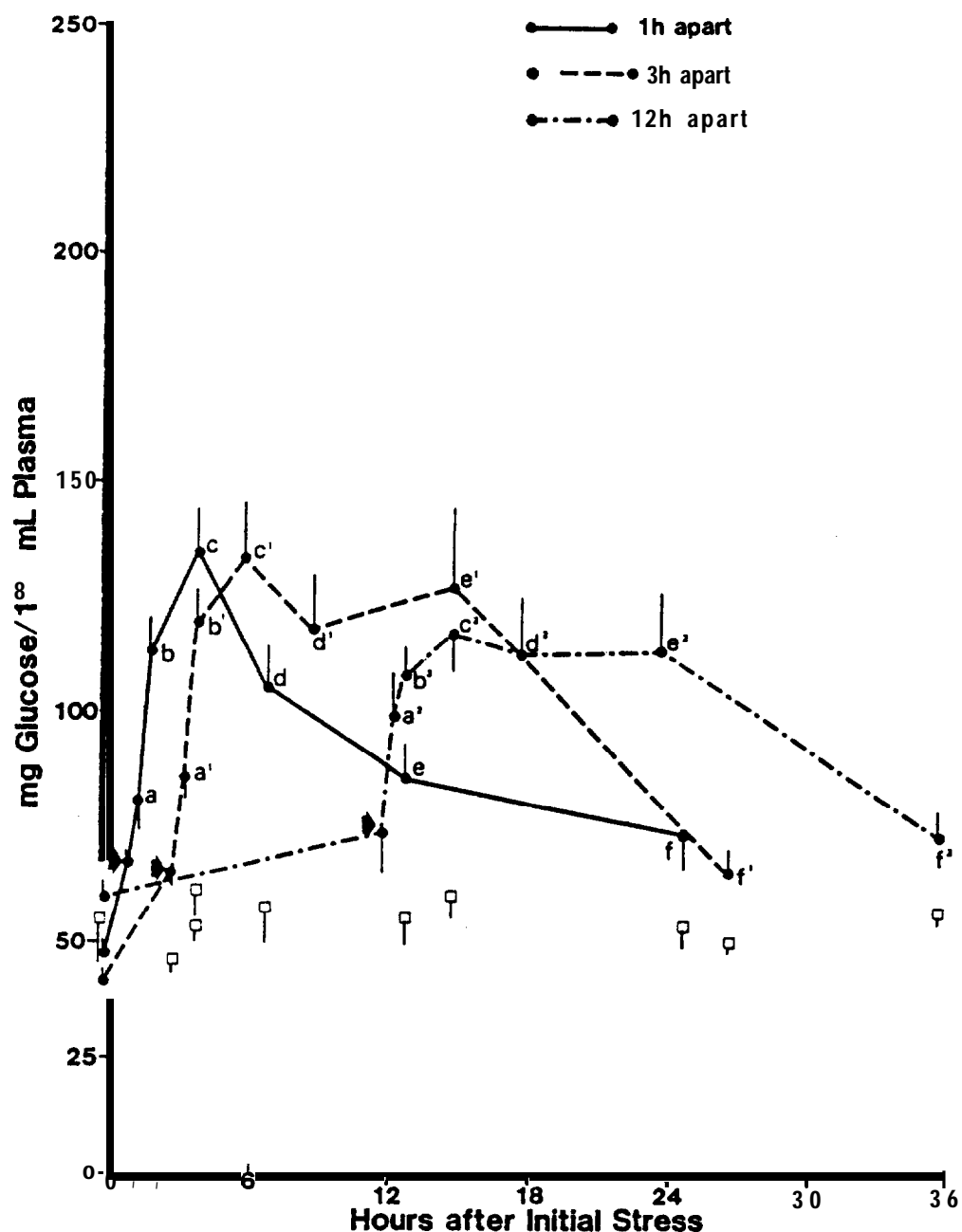
Figure 50. Plasma glucose (mg/100 mL +/-SE) in juvenile fall chinook salmon
subjected to two 30-s handling stresses spaced 1, 3, or 12 h apart.
Arrows indicate time of second stress. Sample sizes of n = 12
represent pooled data for duplicate tanks. Open squares are values
for unstressed controls, Mean values for specific time points
after application of the final stress, as designated by letters
on the figure, are ranked as follows from left to right in order
from lowest to highest; means not significantly different are
underlined (Duncan's new multiple-range test at 5%):

| 0.5 h: | $\underline{a \quad a^1 \quad a^2}$ | 6 h: | $\underline{d \quad d^2 \quad d^1}$ |
| 1 h: | $\underline{b^2 \quad b \quad b^1}$ | 12 h: | $\underline{e \quad e^2 \quad e^1}$ |
| 3 h: | $\underline{c^2 \quad c^1 \quad c}$ | 24 h: | $\underline{f^1 \quad f^2 \quad f}$ |
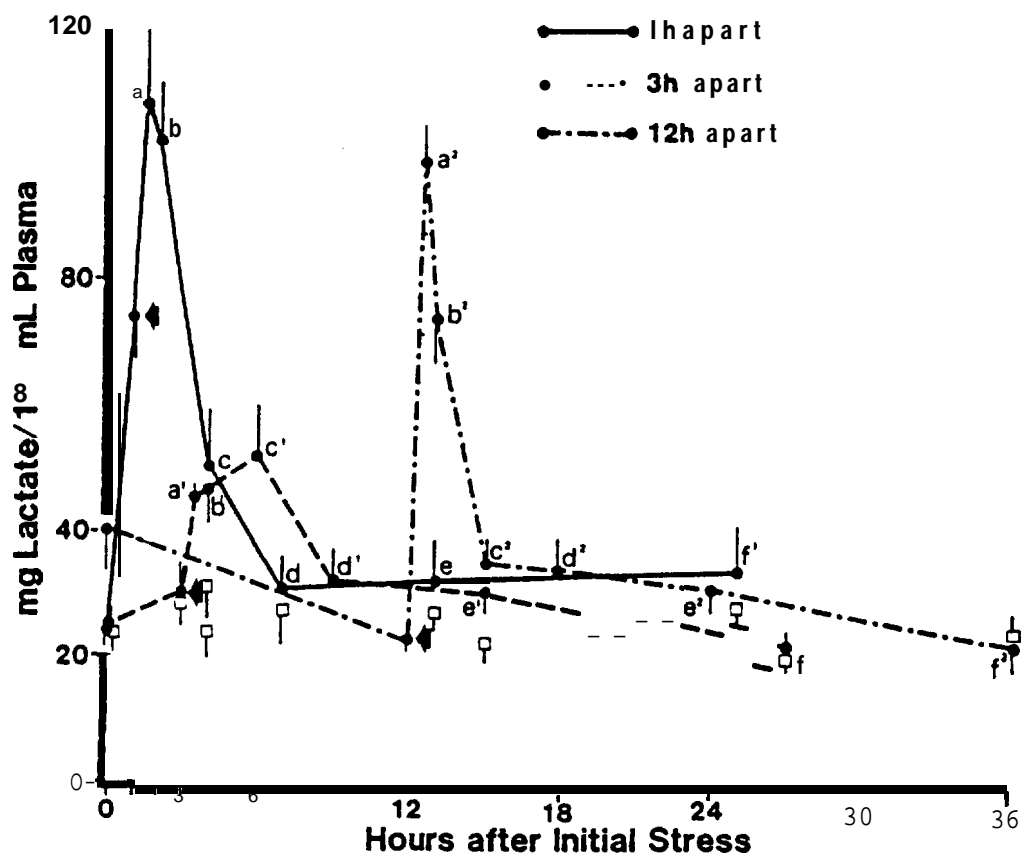
Figure 51. Plasma lactate (mg/100 mL +/-SE) in juvenile fall chinook salmon subjected to two 30-s handling stresses spaced 1, 3, or 12 h apart. Arrows indicate time of second stress. Sample sizes of n = 9-12 represent pooled data for duplicate tanks. Open squares are values for unstressed controls. Mean values for specific time points after application of final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

$$
\begin{array}{llll}
0.5\ \text{h:} & \underline{a^1} & < & a^2 & a \\
1\ \text{h:} & \underline{b^1} & < & b^2 & < & b \\
3\ \text{h:} & \underline{c^2} & & c & & c^1 \\
6\ \text{h:} & \underline{d} & & d^1 & & d^2 \\
12\ \text{h:} & \underline{e^1} & & e^2 & & e \\
24\ \text{h:} & \underline{f^1} & & f^2 & & f \\
\end{array}
$$

about optimum allowable recovery time for fish which have been subjected to physical disturbances, such as they encounter during the collection and transportation procedures. Research is being continued in this area to determine the length of this apparent post-stress "sensitive" period to additional stresses.

Acclimation Temperature and Stress

Experimental design, results, and discussion. Water temperature at McNary Dam varies considerably during the smolt emigration (Fig. 9). It has been shown that water temperature has a profound influence on many physiological functions in fish (e.g., Brett 1979, O'Neill 1980) including their physiological responses to stress (Umminger and Gist 1973; Strange et al. 1977). We examined the effect of acclimation temperature upon the magnitude of physiological responses, and subsequent recovery, resulting from an acute stress to determine if the fish react variously to the collection system due to environmental factors.

Juvenile fall chinook salmon were acclimated to three experimental temperatures (7.5 + 0.5 C, 12.5 + 0.5 C, and 21.0 + 0.5 C) using programmable, cam-operated pneumatic temperature controllers (Golden 1974). In two groups of duplicate tanks of fish acclimated to 12.5 C, temperature was either continuously increased or decreased by 1 $C \cdot d^{-1}$ until the desired temperature was reached, after which the fish were acclimated at this temperature for an additional 2 wk. A third group of fish in duplicate tanks remained at 12.5 C. These temperatures were selected because 21.0 C is close to the maximum encountered by juvenile fall chinook salmon during outmigration in the Columbia River; 12.5 C was the ambient rearing

temperature for the experimental fish, and 7.5 C was the lowest temperature possible with the apparatus.

Throughout the acclimation period and the experiment, fish were held in 350-L tanks at a density of ca. 6 $g \cdot L^{-1}$, having an inflow of 10 $L \cdot min^{-1}$ aerated well water. Up to, but not during, the experiment, the fish were fed daily with Oregon Moist Pellets at ca. 1.5% body weight. After acclimation at the final temperature, fish were subjected to the standard 30-s handling stress and returned to the tank. Plasma samples for cortisol and glucose were obtained (see: Sample collection) prior to the stress and at 1, 3, 6, 12, and 24 h after the stress. Samples for liver glycogen were obtained before the stress and at 6, 12, and 24 h.

Basal plasma cortisol levels were similar for all three temperatures (7.5, 12.5, and 21.0 C) and were relatively low (Fig. 52). Peak cortisol levels at 1 h after the 30-s handling stress were also similar to each other, being 190, 209, and 224 $ng \cdot ml^{-1}$ for the 7.5, 12.5, and 21.0 C groups, respectively (Fig. 52). The only significant differences in plasma cortisol were at 6 and 12 h after stress, where plasma cortisol was slightly higher in the low temperature group. Plasma glucose levels in response to the handling stress were substantially higher in the 21.0 C group than at lower temperatures (Fig. 53). The peak concentration of 113 $mg \cdot dl^{-1}$ at 6 h is more than double those found at the two lower temperatures. Plasma glucose returned to pre-stress levels within 12 h at 7.5 and 12.5 C, but not until 24 h at 21.0 C (Fig. 3). Hepatosomatic indices (HSI, i.e., % liver weight-body weight-1) were highest in the low temperature group, being 1.76, 1.28, and 1.01 % for the low, ambient, and high temperature groups, respectively. There was a decline in HSI in all groups in response to the stress (liver glycogen analyses have not yet been completed at this time).
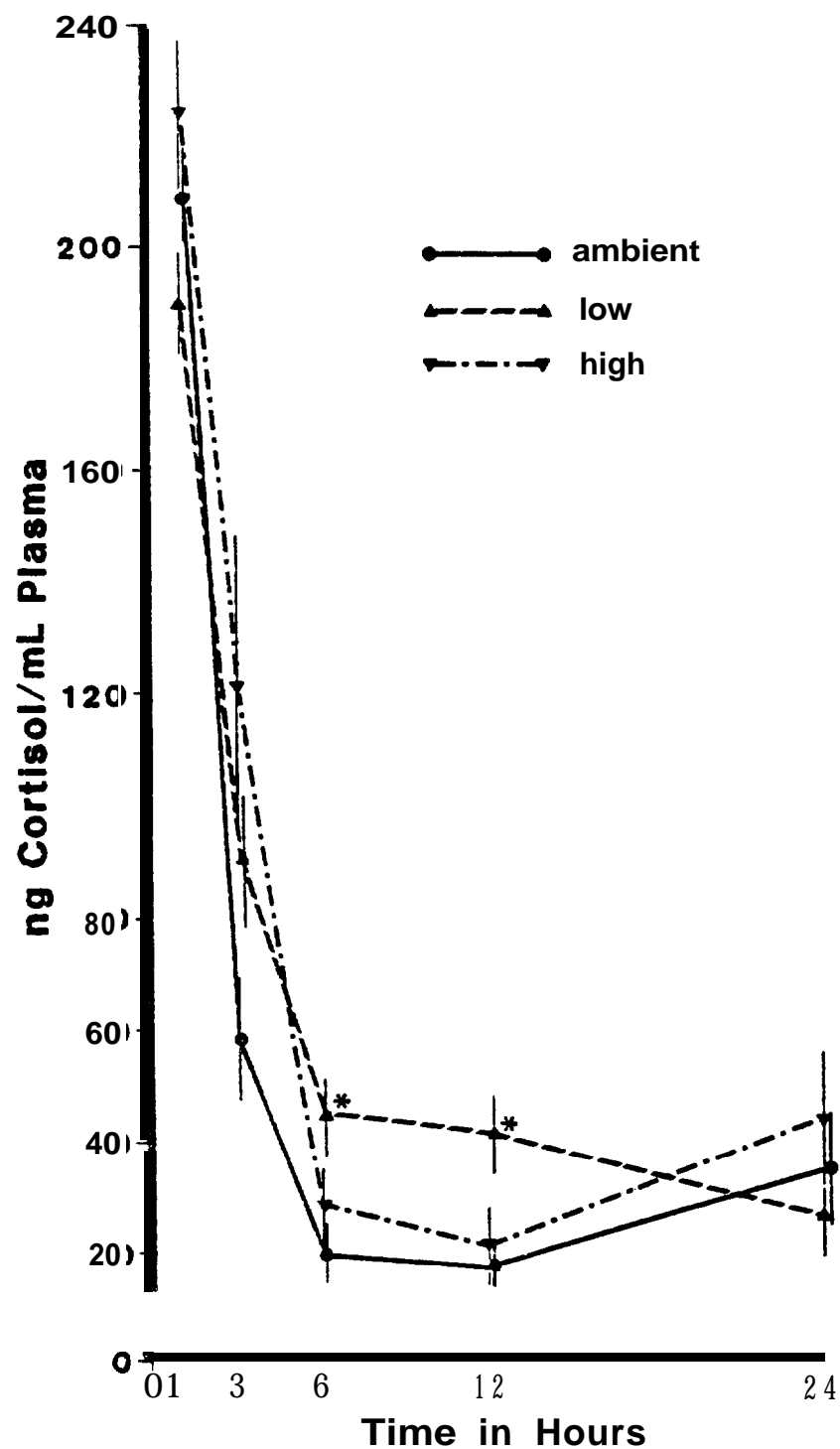
Figure 52. Plasma cortisol (ng/ml +SE) in juvenile fall chinook salmon
acclimated to ambient (12.5 C), low (7.5 C), and high (21.0 C)
temperatures and subjected to a 30-s handling stress. Sample
sizes of n = 12 represent pooled data from duplicate treat-
ments. Values marked with an asterisk (*) indicate a
significant difference from the ambient temperature at
that time point (Duncan's new multiple-range test at 5%).
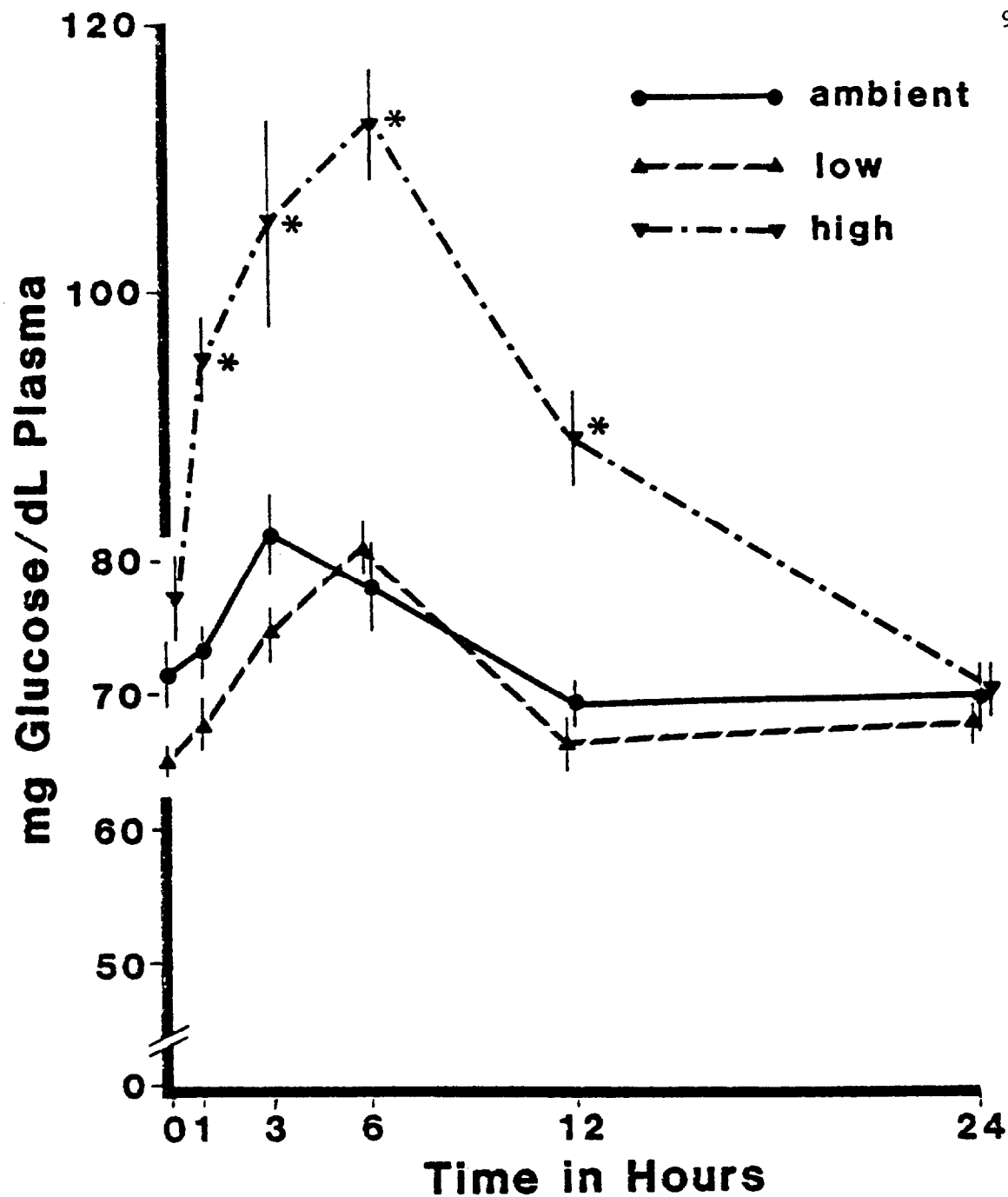
Figure 53.   Plasma glucose (mg/dL ±SE) in juvenile fall chinook salmon
             acclimated to ambient (12.5 C), low (7.5 C), or high (21.0)
             temperatures and subjected to a 30-s handling stress.
             Sample sizes of n = 12 represent pooled data from duplicate
             treatments. Values marked with an asterisk (*) indicate a
             significant difference from the ambient temperature at that
             time point (Duncan's new multiple-range test at 5%).

Acclimation temperature did not appear to affect the peak plasma cortisol level after an acute handling stress. The higher levels of cortisol observed at 6 and 12 h probably resulted from a reduction in the rate at which cortisol was cleared from the body at the lower temperature. The significantly increased plasma glucose levels in response to handling at 21.0 C illustrates the combined effect of the handling stress plus the high temperature and are a reflection of the general level of metabolism at this temperature, as compared to the lower temperatures. Thus, we conclude that at higher temperatures there is a greater metabolic cost associated with stress and that performance capacity may be reduced.

Nutrition and Stress Response

Experimental design, results, and discussion. During our first year of study at McNary Dam, we found that many smolts had empty stomachs and, subsequently, that liver glycogen levels were relatively low (see: System Evaluation). Stress can cause metabolic disturbance in fish (Wendt and Saunders 1973, Leach and Taylor 1980) and the nutritional state of a fish might seriously influence the fish's ability to respond to stress encountered in the collection system. We conducted a controlled diet experiment to determine if the type of diet or fasting alters the physiological response to an acute stress.

Groups of fall chinook salmon were reared on three different diets (i.e., Low [l%], normal [7%1, and high [14%] fat diets) for a period of approximately 3 months (April-June 1983) at Abernathy Salmon Cultural Development Center, Longview, Washington. The diets were based on the Abernathy fish food with either 1, 7, or 13% fish oil added, which resulted

in total fat contents of 7, 13, or 19%, respectively. Fish were acclimated at a density of ca. 5 $g \cdot L^{-1}$ in 700-L circular tanks, each receiving a $12-L \cdot min^{-1}$ inflow of 12 C aerated well water. Prior to stress experiments, duplicate groups from each diet regime were fasted for 20 d. At day 20, all groups were subjected to a standard 30-s handling stress and then allowed to recover in their home tank. Samples for assay of plasma cortisol and glucose were taken prior to the stress and at 1, 3, 6, 12, 24, and 48 h; liver samples for glycogen determination for the fish fed normal and high fat diets were obtained before the stress and at 6, 12, and 24 h.

At the time of the experiment, fasted fish were smaller than their fed counterparts for all three of the diet regimes. Also, condition factor ($K = g \times 100.cm^{-3}$) was lower in the fasted fish, being 1.06, 1.08, and 1.10 compared to 1.20, 1.23, and 1.27 in the fed fish for the low, medium, and high fat diets, respectively. Similarly, the hepatosomatic index (HSI) in the fasted fish was lower than that for the fed fish except for those fish on the high fat diet, where HSI was the same for both fed and fasted fish. After the stress, HSI varied considerably across the different treatment groups. Liver glycogen levels in the fed fish were 35, 20, and 23 $mg \cdot g^{-1}$ for the low, medium, and high fat diet groups, respectively (Table 3). Conversely, in the fasted fish, levels were 6, 5, and 4 $mg \cdot g^{-1}$ for the low, medium, and high fat diet groups, respectively, at the start of the experiment (Table 3). This represents a drop in liver glycogen of approximately 80% over the 20-d fast. At 6 h after handling, both medium and high fat diet groups showed decreased glycogen levels of 13 and 16 $mg \cdot g^{-1}$, respectively, and remained below initial levels for the following 24-h period (Table 3). Liver glycogen levels in the fasted fish remained

Table 3. Plasma cortisol $(ng \cdot mL^{-1})$, plasma glucose $(mg \cdot dL^{-1})$ and liver glycogen $(mg \cdot g^{-1})$ +SE in fed and 20-d fasted juvenile chinook salmon after receiving low (7%), normal (13%), or high (19%) fat diets and then subjected to a 30-s handling stress. Sample sizes of n = 9-11 represent pooled data from duplicate treatments.

Time (h)		Fed Fish			Fasted Fish		
		Low Fat	Normal Fat	High Fat	Low Fat	Normal Fat	High Fat
0	Cortisol	66 +11	41 +9	49 +7	9 +3	20 +5	19 +8
	Glucose	82 +3	78 +3	83 +2	68 +3	67 +3	61 +2
	Glycogen	35 +4	20 +3	20 +3	6 +1	5 +1	4 +1
1	Cortisol	229 +11	204 +17	185 +23	217 +17	216 +13	186 +16
	Glucose	109 +5	100 +5	107 +5	79 +3	80 +3	77 +3
	Glycogen						
3	Cortisol	67 +8	56 +12	78 +12	67 +10	73 +8	59 +11
	Glucose	112 +5	106 +8	132 +9	83 +3	98 +3	85 +4
	Glycogen						
6	Cortisol	38 +7	58 +13	89 +14	80 +17	63 +24	36 +9
	Glucose	89 +4	105 +7	144 +3	102 +10	103 +9	97 +8
	Glycogen		13 +2	16 +3		4 +1	5 +2
12	Cortisol	111 +16	96 +15	37 +7	45 +5	44 +13	32 +8
	Glucose	88 +3	101 +5	115 +6	77 +4	93 +11	74 +4
	Glycogen		15 +4	16 +2		6 +1	5 +1
24	Cortisol	25 +6	64 +9	42 +11	25 +7	10 +5	18 +10
	Glucose	88 +3	99 +5	116 +9	70 +3	75 +4	81 +7
	Glycogen		11 +3	19 +2		6 +1	5 +1
48	Cortisol	33 +10	36 +13	32 +8	23 +3	29 +13	29 +10
	Glucose	78 +3	80 +4	84 +4	58 +4	65 +3	69 +3
	Glycogen						

low and did not change as a result of the handling stress. Basal levels of both plasma cortisol and glucose were lower in the fasted fish for all three diet groups (Table 3). However, plasma cortisol levels subsequent to the handling stress were similar in both the fasted and fed fish (Table 1), except for an unexplained increase in the low and normal fat diet groups at 12 h. Conversely, plasma glucose levels were noticeably higher in the fed fish than in the fasted fish, particularly in the high fat diet group where the peak glucose level of 144 $mg \cdot dl^{-1}$ occurred at 6 h (Table 3).

As expected, the handling stress caused a decline in liver glycogen in fed groups but not in the fasted groups in which glycogen levels were already low. The unusually high basal liver glycogen content in the low fat diet group was probably due to the higher proportion of carbohydrate added to the diet formulation in place of the fat. Although basal levels of plasma cortisol appeared to be reduced in fasted fish, neither fasting nor type of diet affected levels of plasma cortisol in response to an acute handling stress. However, plasma glucose levels after handling were greater in fed fish as compared to fasted fish. Moreover, the greatest glucose level in response to the stress exhibited by the high fat group suggests that stored lipids may be more important than glycogen as a source of energy during stress. Thus, speculatively, fish fed a high fat diet prior to release from a hatchery may be better suited for coping with stresses in a new environment, such as when released into the Columbia River for emigration, by being able to more effectively mobilize their energy reserves.

Cortisol and Disease Resistance

Experimental design, results, and discussion. Stress encountered at the dam appeared to elevate plasma levels of cortisol for significant periods of time (see: System Evaluation). It is known that corticosteroids can have an adverse effect on the immune system and disease resistance (see reviews: Baxter 1976, Ellis 1981). Hence, we conducted the following experiment at the Oregon Department of Fish and Wildlife Fish Disease Laboratory, Corvallis, Oregon, to examine the effects of elevated plasma cortisol titers on juvenile salmons' disease resistance. Unfortunately, coho salmon were the only fish available at the time of this experiment; however, we believe that the results are illustrative of mechanisms found in salmonids in general. Reagent hydrocortisone (i.e. cortisol) was dissolved in molten (45 C) cocoa butter at a concentration of 40 $mg \cdot ml^{-1}$ and 0.1 ml was injected intraperitoneally into each fish after the procedure of Pickering and Duston (1983). The molten cocoa butter solidifies immediately upon injection and forms a bolus which slowly leaches cortisol into the fish's system, resulting in elevated plasma cortisol levels for at least 7 weeks (Maule et al., unpublished data). The concentration of cortisol used was selected based on a dose response study comparing 20, 40, and 80 $mg \cdot ml^{-1}$ cortisol in cocoa butter. The 40 $mg \cdot ml^{-1}$ dose resulted in plasma cortisol Levels close to the lowest levels seen in fish at McNary Dam.

After anesthetization in 50 $mg \cdot ml^{-1}$ MS-222, 5 groups of 25 fish each were injected with cortisol-cocoa butter and put in 64.5 L cuboidal tanks with a continuous supply of aerated, fish-pathogen-free well water, preheated to 15 C. Additionally, 3 groups of 25 fish were injected with

molten cocoa butter and placed in similar tanks. Eleven days after
injection, 2 groups of cortisol-injected fish and 2 groups of cocoa
butter-injected fish were inoculated with Vibrio anguillarum (see: Disease
challenge). The remaining groups of fish were used as controls and to
monitor plasma cortisol levels. All groups were checked daily for
mortalities, and Vibrio anguillarum was isolated from the kidneys of all
mortalities. The disease challenge was terminated 14 d after inoculation
and all survivors were bled for plasma cortisol determination and were
examined for the presence of a cocoa butter bolus in the peritoneal cavity.
Percent mortality and mean of the time to death were calculated for the
replicated groups.

The intraperitoneal cortisol injections resulted in plasma cortisol
levels equivalent to those in fish sampled from the collection and
transportation system and resulted in significantly higher mortalities
than controls when exposed to Vibrio anguillarum (Fig. 54). There was no
significant difference in the mean of the times to death for the two groups,
3.6 + 0.1 and 3.4 + 0.1 days for the cortisol-treated and control groups,
respectively. Cortisol is known to cause the lysis of white blood cells
in the mammalian system (Ellis 1981), and we have seen reduction in
the numbers of white blood cells which may be the result of the stress-
induced plasma cortisol elevation in fish entering the collection system
at McNary Dam (Figs. 15, 16, 29, 40, and 41). We believe that the
connections between stress, cortisol, immunocompetence, and disease
resistance may be affecting the long-term survival of salmonids in the
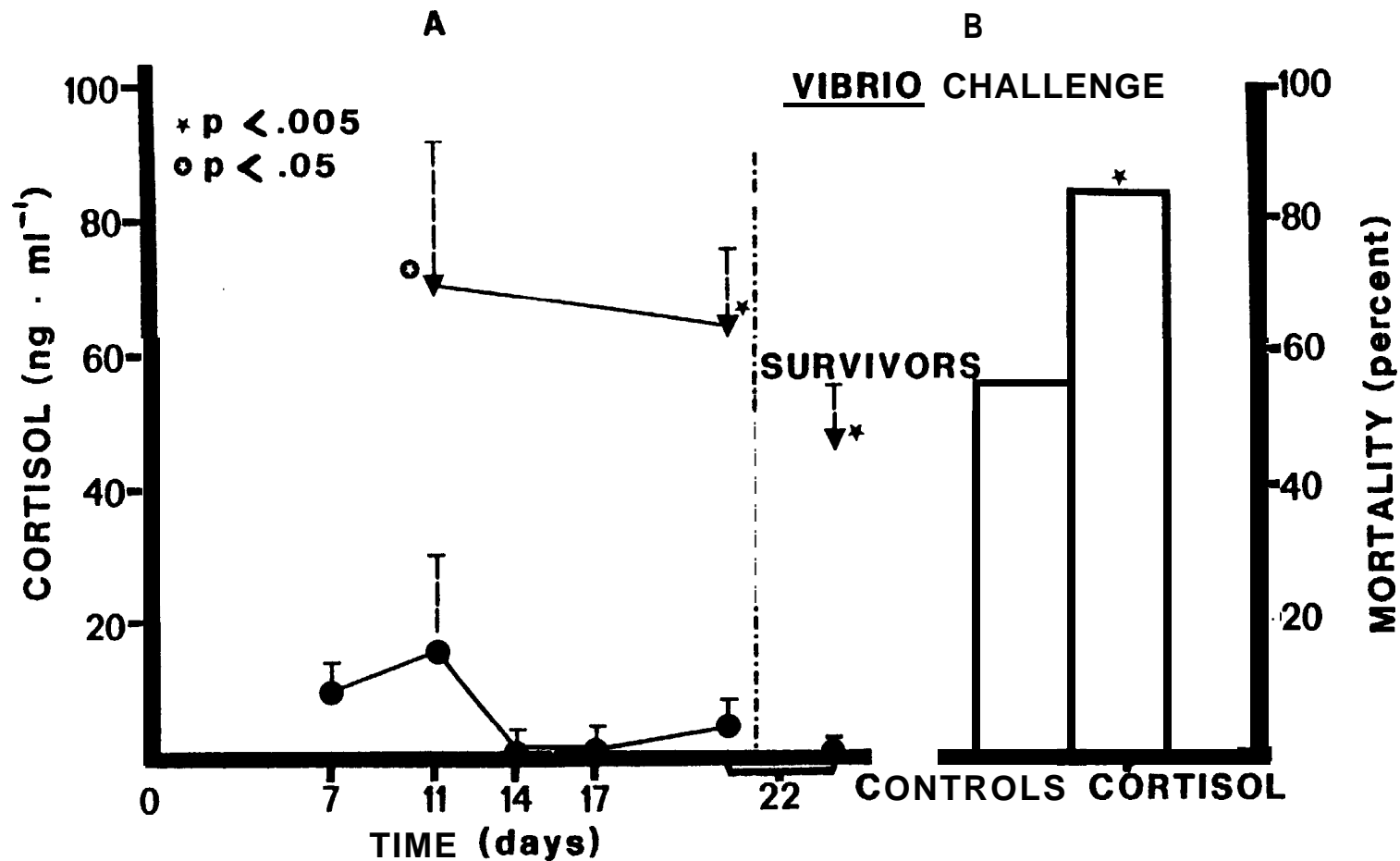Columbia River.

Figure 54. A. Plasma cortisol levels of juvenile coho salmon which were intraperitoneally injected with cortisol in molten cocoa butter (▼) and then injected with just cocoa butter (●). B. Mortality and plasma cortisol levels of coho injected as in A and subsequently exposed to <u>Vibrio anguillarum</u>.

Stress and Swimming Performance

Experimental design, results, and discussion. A smolt's capacity to swim
quickly or for a sustained time may be impaired if the fish is in a stressed
condition. This may compound the already reduced swimming efficiency of
fish consequent to smoltification (Flagg et al. 1983). This impaired
performance capacity may then result in reduced survival when the fish
encounters predators or physical obstructions in the water. Experiments are
being conducted at the Marine Science Center to examine the effects of
stress on critical swimming speed, the maximum speed that a fish can
maintain for a given length of time, and fatigue time, the length of time
that a fish can maintain a given swimming speed. These variables are
defined by the structure and use of the swimming tube in which they are
measured. The portion of our tubes in which fish swim was .076 m diameter
by 1.5 m in length (.25 x 3.0 ft) with screening at both ends to contain the
fish. The inflow end of the tube also had a baffling screen to eliminate
turbulence. The flow meter was positioned on the inflow end of the tube
behind the barrier screen, so as not to interfere with fish in the tube, but
it was calibrated to the flow in the swim portion of the tube. Water inflow
was through a 36 cm (ll/4 inch) PVC pipe, and velocities in excess of
105 $cm \cdot s^{-1}$ could be achieved.

In these experiments, critical swimming speed was measured by
increasing the water velocity in the tube and noting the velocity at which
the fish stopped swimming. The swim tubes were calibrated to the fall
chinook prior to the experiments; that is, we determined the length of time
required to acclimate fish to the tube (18-24 h), optimum number of fish to

be used in a trial (3 fish), magnitude of increments (10 $cm \cdot s^{-1}$) and length of time at each water velocity increment (5 min), and the minimum water velocity at which the fish must actively swim (25 $cm \cdot s^{-1}$).

In the critical swimming speed experiments, 3 fish were allowed to acclimate in each tube overnight with the water velocity at 5 $cm \cdot s^{-1}$. Stress was applied by draining the water from the tube for 30 s (similar to 30 s in a dipnet, out of water). Fish which were not stressed served as controls, and other fish were stressed 1, **2,** or **3 times** with 1 h delay between stresses. After each stress, the water velocity was maintained at **5** $cm \cdot s^{-1}$ until the next stress or until the swimming trials which were conducted immediately after the last stress or at 1, 3, 6, 12 or 24 h after the last stress. A swimming trial consisted of increasing water velocity immediately to 25 $cm \cdot s^{-1}$ and maintaining it there for 30 min. This 30 min is apparently necessary for fish to fully shift into a swimming mode and results in less variability in the data (Brett 1965). Water velocity was then increased 10 $cm \cdot s^{-1}$ every 5 min, and the time and water velocity at which each fish stopped swimming was noted. Critical swimming speed (CSS) was calculated as:

$$css = V_{max-I} + \frac{(I)(t)}{T_{max}}$$

where: T_{max} = maximum length of time at any water velocity, here equal to **5 min**

           V_{max-I} = maximum water velocity achieved and maintained for T_{max}

           t = the length of time the fish **swam** at the maximum water velocity achieved (Vmax)

           I = increment at which water velocity was increased, here equal to 10 $cm \cdot s^{-1}$.

For example, if a fish was able to swim for 2 min at 50 $cm \cdot s^{-1}$, then

V_{max-I} = 40 $cm \cdot s^{-1}$, t = 2 min

and CSS = 40 $cm \cdot s^{-1}$ + $\dfrac{(10\ cm \cdot s^{-1})\ (2\ min)}{5\ min}$ = 40 $cm \cdot s^{-1}$

Experiments to determine fatigue time were conducted in much the same way as the critical swimming speed experiments. Three juvenile fall chinook were acclimated overnight and stressed either 0, 1, 2, or 3 times as above. Fatigue trials were run at 0, 1, 3, 6, 12, and 24 h after the last stress and consisted of immediately increasing the water velocity to 25 $cm \cdot s^{-1}$ for 30 min. Water velocity was then increased to 60 $cm \cdot s^{-1}$ and the time at which each fish stopped swimming was noted. The high water velocity was selected as it is approximately the critical swimming speed of non-stressed fall chinook. (The fatigue time experiments are in progress and no data are presented here.)

Critical swimming speed of these stressed fish tends to be below that of unstressed acclimated fish (Fig. 55), indicating that stress can reduce fall chinooks' swimming performance. The results of these experiments are highly variable and do not show differences in swimming performance of fish stressed one, two, or three times with 1 h between stresses. Conceivably, the fish were recovering during the 1 h between stresses, eliminating any reduced swimming capacity caused by the earlier stress. Completion of this series of experiments, which include tests for the time required for a fish swimming at a constant speed to become fatigued, and the assay of plasma lactate and glucose will hopefully elucidate the stress-swimming capacity interaction.
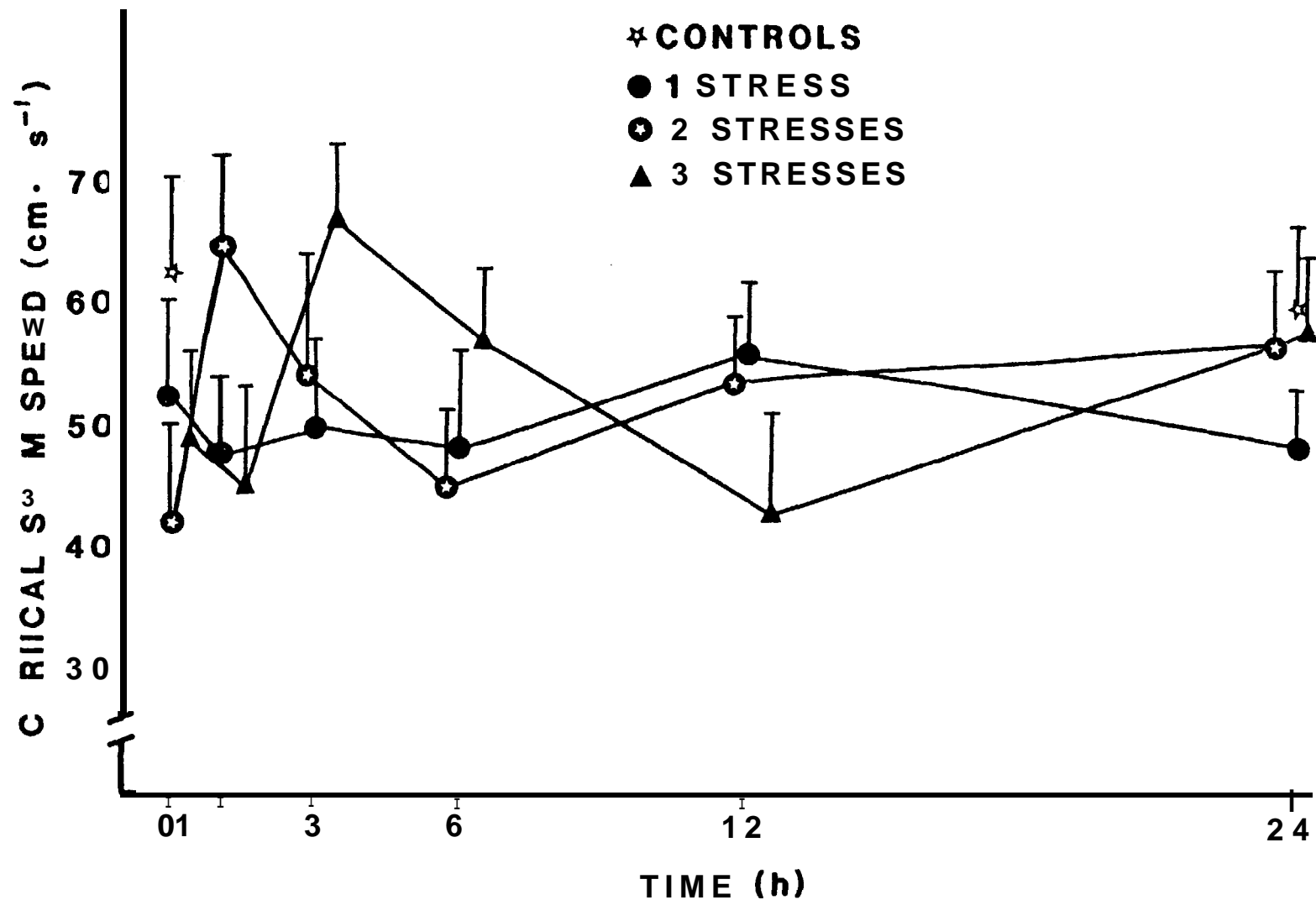
Figure 55. Critical swimming speed, i.e., maximum speed at which fish can maintain position in a flow of water, of juvenile fall chinook salmon either not stressed (controls) or stressed one, two, or three times with 1 h between stresses. The stress consisted of drawing the water from the swimming tube so that the fish were out of water for 30 s. Points are the means + SE for 12 fish.

SUMMARY

Collection System

The results indicate that fall and spring chinook smolts are stressed as they are manipulated by the elements of the collection and transportation sytem at McNary Dam. These stresses are physiologically characterized by transient increases in plasma cortisol without increases in interrenal cell nuclear diameters, indicating that the overall stress is acute rather than chronic. Stress-induced metabolic disturbances are evidenced by increases in plasma glucose and decreases in hepatic glycogen; however, the variable nutritional status of emigrating fish could greatly affect the changes in these indices. There is also a wide variability in hematocrit and leucocrit responses, and there appears to be a latent depression in WBC count within 24 to 48 h after the stress of collection at McNary Dam or transport to Bonneville Dam. The results of the secondary stress challenges and saltwater challenges suggest that the smolts' performance is impaired by the stresses of the collection and transportation.

The patterns of plasma cortisol levels in spring and fall chinook smolts taken from various elements in the system (Figs. 3-6, 23, 24) and of fish exposed to a secondary stress (Figs. 20, 21, and 31) indicate that the elements of the collection system have cumulative effects on the fishes' physiological responses to stress. Therefore, any modification in the collection system that reduces stress at one point in the system should lessen the total physiological impact on the fish. Although we have not verified that there is a direct, linear relationship between the degree of stress and increase in plasma cortisol titers, it appears that the most

stressful part(s) of the collection system is between the gatewell and the bar-sorter (Figs. 5, 25 and 26).

Prior to the 1983 smolt emigrations, several modifications were made in the collection system, which resulted in increased water flow from the collection flume through the vertical pipe and to the upwelling box on the downstream side of the dam (Delarm et al. 1984). The primary objective of these modifications was to prohibit the build-up of adult American shad at the end of the flume, as their presence there late in the summer appeared to inhibit the movement of fall chinook smolts into the vertical pipe. The modifications succeeded in flushing the shad through the system (Brad Eby, personal communication), and this may have reduced the stress experienced by fall chinook late in the run. Plasma cortisol levels in fish sampled during the late run, 1982, were considerably higher than those of fish sampled earlier in the run (Fig. 3). However, in 1983, after the modifications increased flow, plasma cortisol levels were not elevated later in the run. We speculate that the increased water velocity through the system may have decreased the stress experienced by smolts at all times of the run, as plasma cortisol levels were lower in all aspects of the collection system in 1983 as compared to 1982 (Figs. 4-6, 20, 21, and 32). We reason that the increased flow through the vertical pipe may have flushed smolts to the upwelling box before they were more severely stressed by swimming against the flow. Additional support of this interpretation is that there were no differences in plasma cortisol levels in fish transported and held at Bonneville Dam in 1983 compared to 1982 (Figs. 34 and 35). If the difference in plasma cortisol levels of fall chinook at McNary Dam between 1982 and 1983 were the result of differences in the river environment or

variability in the fish, responses of fish transported to Bonneville Dam should have paralleled those in fish at McNary Dam. However, this was not the case, indicating that the differences seen at McNary Dam may have been the result of modifications in the collection system.

Osmoregulatory ability was reduced late in the run in 1982 and again late in the run in 1983 (Figs. 18, 19 and 42), probably the result of the increased water temperatures as the runs progressed. We found that fall chinooks' plasma cortisol response to stress was independent of acclimation temperature (Fig. 52) but that plasma glucose response was elevated at the highest temperature (Fig. 53). This suggests that at higher temperatures, a greater metabolic cost accompanies the response to stress. Osmoregulatory stress placed energetic demands on fish, and the increased temperatures may have interfered with smolts' ability to efficiently use the energy required to perform a variety of tasks. The physiological indices which normally show changes in energetic demands placed on fish (hepatic glycogen and plasma glucose) were highly variable in both years (Figs. 11, 12, 25-27, and 38), perhaps the result of variable nutritional states of the fish.

Juvenile salmon usually avoid bright light by staying in shadows or deep water during the day (Hoar 1958, All 1959). The sudden exposure to **bright sunlight when fish reach the upwelling box and continued bright** light, both natural and artificial, throughout the fishes' stay in the holding facility may also be stressful. We will be testing this possibility during the 1984 sampling by covering most of the system, from upwelling box to raceway, with black plastic or opaque black netting.

We do not believe that the raceways themselves were particularly stressful. The changes in physiological indices of stress in fish after a short time in the raceway were latent responses to stresses of the collection procedures. It appears that the post-collection recovery time of 24 to 48 h is optimal, given the operational necessities at the dam and the rates at which the clinical indices of stress return to baseline. However, emigrating smolts can be held too long as evidenced by reduced osmoregulatory capacity (Figs. 18 and 24) and disease resistance (Table 1) in fish held for 7 to 8 d. Aggressive behavior is apparently maintained in migrants (B. Olla, National Marine Fisheries Service, personal communication); social interactions encountered while in the raceways could account for the delayed stress of being held in the raceways.

The maximum density at which fish were held in raceways (0.5 $lbs \cdot gal^{-1}$) was not excessive. In all but one of our tests, plasma cortisol levels of fall and spring chinook were reduced within 24 h of entry into the raceway, independent of fish density, at or below the maximum (Figs. 3-6, 23, 24, and 32).

The anesthetization, handling, and marking of smolts at McNary Dam apparently did not cause stress in addition to that experienced by fish going directly into the raceways (Fig. 33), as evidenced by plasma cortisol changes. **This does** not **imply, however, that the marking procedure does not** Impair the performance abilities of the fish. Care must be taken to ensure that marked fish have a reasonable time to recover from the procedure before transport or release into the river (ca. 24 to 48 h). We will again examine the effects of marking in 1984.

Transportation System

The most stressful event in the transportation system appears to be loading the fish into the transport vehicle (Figs. 34-36, and 44). Immediately after loading, fish appeared to be stressed, independent of the density of fish in the tank. However, higher densities enroute did appear to affect the fishes' ability to recover from the stress of loading (Figs. 35, 36 and 44). The maximum allowable density of fish in a transport truck (0.5 $\text{lbs} \cdot \text{gal}^{-1}$) was not excessive, but exceeding this level might interfere with the enroute recovery (Fig. 44). Moreover, transporting fish at less than the maximum density allowed greater recovery from the stress of loading.

Fall chinook smolts removed from the transport vehicles at Bonneville Dam recovered from the stress of the transport and sampling procedures within 24 h, as evidenced by plasma cortisol levels (Figs. 34 and 35). Moreover, these procedures elicited uniform responses in fish, with no significant variation occurring between years or within the run (Figs. 34 and 35), indicating that variation in the environment or the fish did not affect the plasma cortisol response. This lack of variation in plasma cortisol response appears to indicate that before the fish were transported, they had recovered from the stresses of collection at McNary Dam, which do show within- and between-years variation (Figs. 3-6).

Fall chinook smolts transported to Bonneville Dam did show reduced osmoregulatory ability late in the run (Fig. 42), again suggesting that environmental temperature is a critical factor in performances such as osmoregulation. This could mean that the ability of fish to perform any

energy-demanding task was more impaired by the combination of stress and high temperature than by either factor alone.

The results of the disease challenge (Table 1) and saltwater challenges (Figs. 19 and 42) suggest that a l-d recovery period after transport may be optimal. However, out of necessity, we handled these fish prior to the tests. We do know that upon arrival at Bonneville Dam, fish were in the process of recovery (i.e., have lower plasma cortisol levels) from the stress of being loaded into the truck or barge. We do not know the nature of fishes' response to the release procedures; however, we speculate that fish released from the barge by draining the tanks directly into the river would not be stressed as seriously as fish released from a truck through a 100-m flexible tube. Moreover, we speculate that the disorientation of being returned to the river after l-2 d in the collection and transportation system may compound the stress of the physical disturbance of release. Smolts primarily emigrate at night (Hoar 1958, All 1959) and, consequently, it seems reasonable that transporting and releasing fish at night might be less stressful and cause less disorientation upon release.

We have presented data on the effects of collection and transportation on short-term dynamics of physiological indices of stress and performance capacities in juvenile salmonids in the Columbia River. It appears that these manipulations do not cause serious, short-term dysfunction for the fish; however, the question remains as to long-term effects that the collection and transportation system may have on smolt survival. We believe that there are additional management options and system modifications which should be tested.

CONCLUSIONS

(interim, to be finalized in 1985)

Juvenile fall chinook were stressed by the collection system at McNary Dam.

The elements of the collection system had cumulative effects on the fishes' response to the system.

Changes in the collection system between 1982 and 1983 decreased the total stress experienced by fall chinook collected.

There were seasonal variations in some physiological responses to stress, probably the result of changes in the environment.

The maximum raceway density of 0.5 $lbs \cdot gal^{-1}$ was not excessive.

Fall chinook which were anesthetized, handled, and marked were no more stressed than fish which just went through the collection system, but required a day's recovery time before transport or liberation.

Optimum length of time for fall chinook to recover from the stresses of collection is 24 to 48 h.

Loading fish into the transport vehicle was the most stressful event in the transportation procedure.

The transport vehicles were not stressful and the fish showed some recovery from the stress of loading while enroute.

The maximum transport loading density of 0.5 $lb \cdot gal^{-1}$ was not excessive.

ACKNOWLEDGEMENTS

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